REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	Aug 96	

4. TITLE AND SUBTITLE
The Effect of Room Temperature Storage on the in Vitro Storage Characteristics of CPDA-1 Packed Red Blood Cells

5. FUNDING NUMBERS

6. AUTHOR(S)
Jean P. Ruddell

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Bowling Green State University

8. PERFORMING ORGANIZATION REPORT NUMBER
96-059

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
DEPARTMENT OF THE AIR FORCE
AFIT/CI
2950 P STEET, BLDG 125
WRIGHT-PATTERSON AFB OH 45433-7765

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS

15. NUMBER OF PAGES
81

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to ***stay within the lines*** to meet ***optical scanning requirements***.

Block 1. Agency Use Only *(Leave blank)*.

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C	- Contract	**PR**	- Project
G	- Grant	**TA**	- Task
PE	- Program Element	**WU**	- Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. *(If known)*

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

- **DOD** - See DoDD 5230.24, "Distribution Statements on Technical Documents."
- **DOE** - See authorities.
- **NASA** - See Handbook NHB 2200.2.
- **NTIS** - Leave blank.

Block 12b. Distribution Code.

- **DOD** - Leave blank.
- **DOE** - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.
- **NASA** - Leave blank.
- **NTIS** - Leave blank.

Block 13. Abstract. Include a brief *(Maximum 200 words)* factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code *(NTIS only)*.

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

THE EFFECT OF ROOM TEMPERATURE STORAGE ON THE IN VITRO STORAGE CHARACTERISTICS OF CPDA-1 PACKED RED BLOOD CELLS

Jean P. Ruddell

A Thesis

Submitted to the Graduate College of Bowling Green
State University in partial fulfillment of
the requirements for the degree of

MASTERS OF SCIENCE

August 1996

Committee:

Stan Smith, Advisor

Lloyd E. Lippert, Research Advisor

Judy Adams

Lee Meserve

ABSTRACT

Lloyd E. Lippert, Advisor

Packed red blood cells (PRBC) which reach temperatures exceeding 10°C are generally discarded. Little data exist on the degree of accelerated metabolism and increased hemolysis of PRBC allowed to warm for more than a few minutes. Twenty four CPDA-1 PRBC units were pooled in eight groups of three. Each pool was divided into two test units and a control unit. Test units were warmed to 25°C for 24 hours either at day 6 or day 20; controls were maintained at 1-6°C. *In-vitro* storage characteristics were evaluated weekly and prior to warming. Sterility was evaluated at day 35. Data were analyzed by ANOVA. Control units had higher ATP and glucose, less hemolysis, and equivalent morphology to the warmed test units at day 35. Warmed units had adequate ATP and glucose, equivalent hemolysis, and better morphology at day 28 than day 35 control units. With the exception of ATP, test units at day 28 were equivalent to or better than controls at day 35. In the day 28 warmed units, ATP exceeded 1.5 μmole/g hemoglobin (Hgb). No bacterial growth was detected despite repeated sampling. It appeared that a day of 25°C storage of CPDA-1 PRBC accelerated aging equivalent to a week of conventional storage at 1-6°C. It did not appear to matter whether the PRBC were warmed at day 6 or day 20. Medical directors may find this information useful in logistically difficult circumstances.

This work is dedicated to God who created all the wonders of the world.

ACKNOWLEDGMENTS

I wish to acknowledge all those people who lent their support, guidance, and effort toward the successful completion of this project. Sincere appreciation is expressed to my advisor, Dr. Lloyd E. Lippert, for his direction and encouragement throughout this research project. Colonel Hess and Major Bacock for their input and guidance. I would also like to thank Claudia Derse-Anthony, MT(ASCP) and Mike Mechling MLT(ASCP), SBB for their assistance in teaching me all the necessary laboratory techniques. Special thanks to my committee members-Drs. Stan Smith, Lee Meserve, and Judy Adams-for their helpful suggestions and criticisms throughout the development of this thesis, Dr. Kalman Salata and Lt Col Mike Fitzpatrick for their editorial comments and suggestions, and my parents who raised me to be the person that I am today. Sincere gratitude is also expressed to my husband Brent whose patience and understanding gave me the strength to endure.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

The American Association of Blood Banks (AABB) and the Food and Drug Administration (FDA) have established guidelines for the storage and preservation of packed red blood cells (PRBC). According to AABB Standards G1.300 and G2.000, PRBC must be stored between 1 - 6°C and shipped between 1 - 10°C.[1] However, the AABB Technical Manual states "Blood exposed to temperatures above 10°C is not necessarily unsuitable for transfusion."[2] The FDA sets the same standards: 21 CFR 640.2 (e)(3) indicates that blood may not be issued unless it has been stored at 1 - 6°C or maintained at 1 - 10°C during shipping. Section 21 CFR 640.4 (I) indicates that blood should be placed in storage at 1-6°C as soon as platelets are prepared.[3]

Occasionally, units of blood may be left unrefrigerated, alarms may be ignored, or shipments may be delayed and units may reach temperatures above 10°C. The destruction of these units may not be necessary. Do units of PRBC left at temperatures that exceed FDA and AABB requirements for 24 hours remain viable if returned to normal storage temperatures? This question has not been answered.

These standards were established after years of research with many preservative solutions and storage temperatures. Since RBC begin to degrade as soon as they leave the body it was important to find a preservative that maintained cellular integrity and a temperature that would slow RBC metabolism.[4]

Blood preservation was not of concern in the earliest blood transfusions because, either the donor and recipient were physically connected by vein-to-vein anastomosis or the time between collection and transfusion was limited to avoid clotting. However, as

transfusion evolved, storage and preservation became important issues. Citrated blood was first successfully used at Mount Sinai Hospital in New York City in 1914.[5, 6] When sodium citrate was used as an anticoagulant it prevented clotting, but nothing was added to prevent the deterioration of the RBC. The search for a preservative solution had begun. Between World War I and World War II it became evident that the use of preserved blood in wartime would make a significant difference in its availability to wounded soldiers.[7] During this time it was found that the addition of 0.3-3.0% dextrose to the citrate solution would inhibit hemolysis and lengthen survival of RBC in the recipient. Preserved blood had now been used for transfusion but there was still uncertainty about shipping blood to the front lines. [8]

Although the British Medical Service had transported blood during World War I it was still the prevailing belief that RBC could not withstand transportation and would rupture with the slightest trauma. A group at the State University of Iowa conducted a study to prove that preserved blood could be transported without causing an appreciable increase in hemolysis. In their experiment the RBC in glass bottles were transported in milk cans, covered with quilts, and re-iced every 24 hours. Twenty RBC units were transported by plane and twenty by ambulance. There was no significant increase in hemolysis either before or after transport. All of the RBC units were transfused without incidence. Now that it had been demonstrated that RBC units could be preserved and transported the hunt was on for the best preservative solution.[8]

At the Conference on Transfusion Equipment and Procedure in August of 1942 it

was recommended to the Armed Forces that preserved blood be used whenever fresh blood was not available.[9] At the subcommittee meeting in May of 1943 Mollison recommended the use of a preservative solution containing citrate, citric acid, and dextrose. These constituents were used in the preservative solution used for blood flown overseas. Alsever devised a solution composed of 0.42% sodium chloride, 0.8% percent sodium citrate, and 2.05% dextrose which was mixed with an equal volume of RBC. RBC preserved in Alsever's solution was administered in field hospitals in France. Blood could be stored in this solution for 20 to 30 days with continuous refrigeration or 18 to 21 days if refrigeration was interrupted for 24 hours.[10] Early in the search for RBC preservatives it was noted that 24 hours at nonrefrigerated temperatures was equivalent to about one week of refrigerated storage and the shelf life of the RBC was simply reduced.

Another solution ACD (acid-citrate-dextrose) was available but was not selected since the Alsever's containers were already under production and sufficient ACD storage studies had not been completed. ACD had several advantages; a smaller volume was necessary, and the RBC were less fragile and tolerated handling and transportation better than in other preservatives. ACD was tested and found to be a superior preservative to Alsever's, and use in the European theater began in April of 1945.[11, 12, 13]

In 1957 ACD was replaced by CPD (citrate-phosphate-dextrose) which was less acidic. Both ACD and CPD are currently approved preservative solutions for 21 day storage. Adenine was approved for addition to CPD by the FDA in August 1978. The addition of adenine to create CPDA-1 (citrate-phosphate-dextrose-adenine) increases the

amount of adenosine diphosphate, which drives glycolysis toward the synthesis of ATP (adenosine triphosphate).[14] Red blood cells generate most of their energy by glycolysis. This glycolytic pathway is anaerobic and called the Embden-Meyerhof pathway. The components of CPDA-1 are used in the Embden-Meyerhof pathway to produce two moles of ATP from one mole of glucose without oxygen.[15] CPDA-1 PRBC units can be stored for 35 days at 1-6°C. CPDA-1 is currently the preservative used in most military blood banks in the United States.[14] Current procedures dictate that a CPDA-1 PRBC unit that is warmed above 10°C be discarded, but there are no data to support this practice.

There have been studies done on the effect of post-phlebotomy 8 hour holding periods on in vivo and in vitro properties of red cells. When a unit of blood is collected it may remain at room temperature for a few hours to allow transport from a mobile site or to process components. In one study autologous units subjected to an 8 hour holding period post-phlebotomy had comparable 24 hour survival levels after 42 days of 1-6°C storage. These units were held between 24° and 28°C and maintained in vitro and in vivo properties similar to those of units held for only 6 hours at room temperature.[16] Another investigation found that an eight hour hold period did not have a detrimental effect on red cells stored in CPDA-2. The eight hour hold may cause a partial reduction in 2,3-DPG (diphosphoglycerate) but appears to aid other in vitro parameters of red cell quality.[17] 2,3-DPG plays an important role in the physiology of RBC; it is depleted during storage but RBC regain the ability to synthesize it after transfusion.[14] These studies have focused on the prestorage room temperature holding period rather than

warming to room temperature during the refrigerated storage period.

The effect of room temperature storage on bacterial growth has also been of great concern. Most transfusion reactions resulting from bacterial contamination are caused by endotoxins produced by bacteria capable of growing at cold temperatures. A septic reaction has a rapid onset and can lead to death, these reactions are extremely rare.[18] It is believed that allowing blood to warm to room temperature will allow rapid growth of bacteria causing the amount of bacteria in a contaminated PRBC unit to be much higher than in a refrigerated PRBC unit. However, one study found that room temperature exposure of contaminated PRBC did not result in accelerated bacterial proliferation over a period of 2 hours. In fact room temperature exposure had a detrimental effect or no effect at all on the growth of bacteria in intentionally inoculated units.[19] It is important to note that these units were intentionally inoculated with viable bacterial cultures.

RBC are collected in a manner designed to prevent contamination. Units of blood are collected in pyrogen free, sterile containers.[20] The phlebotomy site is prepared according to American Association of Blood Banks standards. The arm is scrubbed with a 0.7% aqueous solution of iodophor compound for 30 seconds, followed by the application of a 10% PVP-iodine in a concentric spiral, and a 30 second incubation.[21]

An Investigation on the sterility of blood products by investigators at the Amsterdam Red Cross reported that none of 509 red cell units tested from 1987 to 1990 were contaminated. They annually store 70,000 units for 16-20 hours at 22°C prior to component preparation and had no reported transfusion reactions resulting from bacterial

contamination during the same time period.[22] Because of precautions taken, units of PRBC should not be contaminated; a 24 hour storage at room temperature may have no effect on bacterial contamination.

The question of whether or not PRBC left at >10°C are suitable for transfusion can be investigated by finding the degree to which the storage lesion (changes that RBC undergo during storage) is accelerated when units of PRBC are intentionally left at elevated temperatures. If PRBC units intentionally left at elevated temperatures maintain viability (as determined by in vitro studies) then some units may possibly be saved and used for transfusion. It is difficult and very expensive to collect and process a unit of PRBC, recruit healthy donors, find rare blood types, or get blood to remote locations. When the supply of PRBC is low, the loss of a few units of PRBC because of a short exposure to elevated temperatures could cost a patient their life. The data provided by the present project may aid medical personnel in determining suitability of PRBC units warmed to >10°C. A physician may have more confidence in using the PRBC with a shortened shelf life if he knows the unit is still transfusable.

The FDA and AABB regulations require that each unit of PRBC will have at least 70% survival 24 hours after transfusion.[2] Although blood can be stored for up to 35 days in CPDA-1 preservative solution, RBC go through changes which are called lesions of storage.[2, 21] The storage lesion is defined as a loss of viability and function associated with certain biochemical changes that are initiated when blood is stored in vitro. The changes that occur during storage of RBC include: a decrease in pH, an accumulation of

lactic acid, a decrease in glucose consumption, a decrease in ATP levels, and a loss of red cell function. The loss of red cell function is expressed as a shift to the left of the hemoglobin-oxygen dissociation curve.[14] During the storage of RBC, certain reversible and irreversible changes occur which lead to the failure of some cells to survive post infusion. Some of these changes are closely correlated with ATP decline during storage.[23]

Certain in vitro tests may indicate the viability of a unit of PRBC. These tests are: ATP; 2,3-DPG; cell morphology; pH; glucose; plasma Hgb; Na^+; K^+; lactate; and total Hgb.[21] The results of each of these tests from units of blood warmed for 24 hours can be compared to the results of control units stored at 1-6°C to give an indication of changes that may affect viability.

Testing the ATP levels of stored RBC is often used as a surrogate test for viability measurements. However, viability by definition, is a measure of in vivo red cell survival and can only be measured in vivo.[24] Furthermore, in vivo survival studies are not practical for screening. ATP levels are an indicator of cell viability, not a true measure of viability.[21] Since the loss of viability of red cells has been correlated with the storage lesion, measurement of the effects of the storage lesion may give an indication of the suitability of a unit for transfusion.[14] Survival of RBC has been shown to correlate with ATP levels above 1.5 µmol per gram of Hgb or above 30% of normal.[14, 25, 26] If the ATP levels of the warmed units are comparable to that of the control units and above 1.5 µmol per gram of Hgb, then the ATP levels should be adequate for red cell metabolism and survival during storage.

2,3-DPG modulates the oxygen dissociation curve of hemoglobin. Therefore, many efforts have been made to design preservative solutions that prevent the rapid decline in the levels of this sugar phosphate in PRBC.[27] Red cell 2,3-DPG levels fall to nearly zero after about two weeks of storage in CPDA-1.[26, 28] 2,3-DPG is important in the delivery of oxygen by RBC, and although most of it is lost during storage it is regenerated within 24 hours post infusion.[24, 28] Since the present project is designed to' compare the difference between control samples stored at 1-6°C at the fifth week, and those warmed during storage at the fourth week of storage, 2,3-DPG levels would be of little use, because the levels are near zero long before the fourth week of storage.

Cell morphology also gives an indication of RBC viability. During storage RBC undergo extensive morphologic changes which tend to parallel the decrease in ATP concentration.[29] There is a red cell transformation from discocyte to spheroechinocyte during the storage period.[30] As the cells change from discocyte to spheroechinocyte they lose membrane vesicles and hemoglobin.[31] Since the cells that lose deformability are removed from circulation by the spleen and liver, the number of discocytes in a blood sample would be a good indicator of how well the cells might survive after transfusion.[32] Some hemolysis of red cells is expected during storage. Hemoglobin leaks as part of the process of vesicle extrusion, red cell lysis, and by the lysis of microvesicles.[33] An increase in plasma Hgb in a stored unit is an indication of red cell loss. In a 1977 study designed to provide data in support of licensure applications for CPD supplemented with adenine (CPDA-1), two of three units with plasma Hgb levels above 1000 mg/dL had

poor survival in vivo.[34]

The pH and the glucose level of a red cell unit are indications of red cell metabolism. Small increases in temperature lead to increased glucose consumption, lactate production and lower pH levels.[35] The maintenance of adequate ATP levels is dependent on the maintenance of the glycolytic pathway.[36] Glucose is the normal substrate for energy metabolism in the human red cell. If the glucose in the preservative solution is consumed during the 24 hour warming, ATP levels will peak and then decline below adequate levels. The glucose level will indicate whether the unit has adequate levels to maintain glycolysis to the point of sampling. The pH is decreased due primarily to the build up of lactate, the end product of red cell metabolism.[24]

In-vitro measurements of ATP, red cell morphology, glucose, plasma Hgb, total Hgb, Na^+, K^+, lactate, and pH can be determined to ascertain if cells warmed to 25°C for 24 hours have parameters similar to those of units stored at 1-6°C (Table 1).On day 35 normal values for plasma Na^+ are 112.84±15.40 mmol/L, for K^+ concentration 87.59±22.6 mmol/L, for pH measured at 38°C 6.46±0.05, and glucose should be present.[34] If the ATP, RBC morphology, pH, lactate, Na^+, K^+, glucose, and plasma Hgb of the warmed units are similar to that of the control units at day 35, the warmed unit might be issued for transfusion. The present project was designed to determine whether units of PRBC lose one week of storage life when held for 24 hours at 25°C at any point during storage, meaning that one day at 25°C is equivalent to one week of conventional 1-6°C storage. A physician could adjust the expiration of a PRBC unit to compensate for

the expected accelerated deterioration caused by exposure to ambient temperatures. Data from the present study should provide physicians with the necessary information to determine the maximum outdate for units exposed to temperature >10°C during storage.

Table 1. Normal Values for CPDA-1 PRBC at Day 35

Day 35 CPD plus Adenine PRBC	ATP µmole/g Hgb	ATP Percent of Day 0	Glucose mg/dL	Plasma Hgb mg/dL
Mean	1.93	45.74	74.79	524.59
Standard Deviation	3.74	12.28	55.96	558.02
Standard Error of the mean	.12	2.82	12.84	128.02

These data were used in support of licensure for CPD plus Adenine preservative solution which is now know as CPDA-1. CPD plus adenine has the same composition as CPDA-1.[34]

Donor Screening

Thirty volunteers were tested for ABO and Rh blood type, antibodies (transfusion/pregnancy history), sickle Hgb, osmotic fragility, and Hgb or hematocrit. Blood was drawn, into a sterile 7 mL nonanticoagulated tube (Terumo Venoject, Elkton, MD, code T2045) and a 5 mL EDTA anticoagulated tube (Terumo Venoject, code T2045QS), from each donor from the antecubital vein. Serum was separated from clotted red cells by centrifugation, and used for infectious disease testing and antibody detection. Infectious disease testing was performed at an FDA licensed reference laboratory. Infectious disease testing was negative for all donors. The EDTA whole blood sample was used for all other testing.

Blood typing

A 2-5% suspension of RBC in normal saline was prepared. Two 12x75 mm glass test tubes were labeled A, and B. One drop each of anti-A, and anti-B (Gamma Biologicals, Houston, Texas, code 4-102 and 4-132) was added to the appropriately labeled tube. One drop of RBC suspension was added to each of the two tubes, mixed, and the cells packed by centrifugation at 1000 x g for 20 seconds (Clay Adams Inc., Sero-fuge II, Cat. No. 0541). The red cells were gently resuspended and visually examined for agglutination. Red cell typing was necessary since donor blood was pooled in groups of three. This information was used to schedule donors with the same blood type at the same time.

Antibody Screen

Those donors with a history of transfusion or pregnancy were screened for alloantibodies. Two drops of serum were placed in test tubes labeled I, II, and III. One drop of screening cells I, II, and III (Gamma Biologicals, Houston, TX) was placed in the appropriately labeled test tube. Two drops of low ionic strength medium (Gamma Biologicals, Houston, TX) were added to each test tube. Contents of the tubes were mixed and incubated in a heat block at 37°C for 15 minutes. The cells were packed by centrifugation at 1000 x g for 20 seconds, gently resuspended, and examined for agglutination. The tubes were placed in an automated cell washer and washed 3 times with normal saline. Two drops of anti-human globulin serum (Gamma Biologicals, Houston, TX) were added to each tube and mixed. The cells were packed by centrifugation at 1000 x g for 20 seconds. The red cells were gently resuspended and examined for agglutination. One drop of Coombs control cells was added to each tube and mixed. The cells were packed by centrifugation at 1000 x g for 20 seconds. The red cells were gently resuspended and examined for agglutination. Volunteers with positive antibody screening tests were excluded from the present study.

Osmotic Fragility

An adaptation of the original erythrocyte osmotic fragility procedure as described by Dacie was used to screen volunteers.[37] Commercially available kits (Becton-Dickinson Unopette test 5830 kit, Rutherford, NJ) using the Dacie method were used to determine the osmotic fragility of each sample. Using a capillary pipette a 20 μL sample of RBC

was added, to 10 reservoirs, containing concentrations of saline ranging from 0 to 0.85%. The contents of the reservoirs were mixed and incubated at room temperature for 20 minutes. The reservoirs were emptied into test tubes and the cells separated by centrifugation at 2000 rpm for 5 minutes. The supernatant fluid was pipeted into a 4 mL spectrophotometer cuvette and the absorbance was read at 540 nm (Beckman DU62, Beckman Instruments, Columbia, MO). Percent hemolysis was determined by the following formula:

$$\frac{\text{specimen absorbance - absorbance of 0.85\% saline cuvette}}{\text{absorbance 0\% saline cuvette - absorbance of 0.85\% saline cuvette}}$$

The percent hemolysis was plotted against the percent saline that elicited the hemolysis. The concentrations of saline causing 10 and 50 percent hemolysis were determined and plotted against time.[38, 39] Donors with normal osmotic fragility were selected.

Sickle Cell Screen

A commercially available kit was used for the qualitative determination of hemoglobin S in volunteer's blood (Columbia Calibre, Springfield VA, catalog number K110A). Two mL of working sickle cell reagent was placed in a 12 x 75 mm tube. Twenty µL of whole blood was added to each tube and mixed by inversion. A positive and negative control were included for comparison. The tubes remained at room temperature for 2-5 minutes. Each tube was held in front of a line scale. If the solution was clear and the lines visible, then the test was negative. If the solution was turbid and the lines not visible, then the test was positive.[40] Donors with a positive test were not

used in the study.

Donor Selection

The results of the previous tests were used to select 24 donors. Donors were required to have negative infectious disease testing, a hematocrit $\geq$ 38% or a Hgb $\geq$ 12.5 g/dL, a negative antibody screen, a negative sickle Hgb test, normal osmotic fragility, and were grouped with 3 volunteers of like ABO type. Volunteers were screening was performed two weeks prior to their draw date. Donors were scheduled in two pools of three per day, for four days for a total of 24 donors. Volunteer blood donors who had not donated for at least 56 days and were acceptable by American Association of Blood Banks and the Food and Drug Administration standards were used. Donor questionnaire (Department of Defense form 572) was completed by each volunteer. The blood collection protocol was approved by the Institutional Review Board of the Walter Reed Army Institute of Research. Informed consent was obtained from all volunteers.

Packed Red Blood Cells

A CPDA-1 double bag collection system (Fenwal, Baxter Health Care Corporation, Deerfield, IL, Code 4R6210) was used to collect the donor's blood. The plasma was separated from the red cells by centrifugation at 5000 x g for 5 minutes. The plasma was expressed using a Fenwal Plasma Expresser (code BM-1, Morton Grove, IL) into the single satellite bag, which was removed after the tubing was heat sealed (Hematron Dielectric sealer, Deerfield, IL, model H-1).[41] The final hematocrit was less than 80% for each PRBC unit as determined by spun hematocrit. The amount of plasma

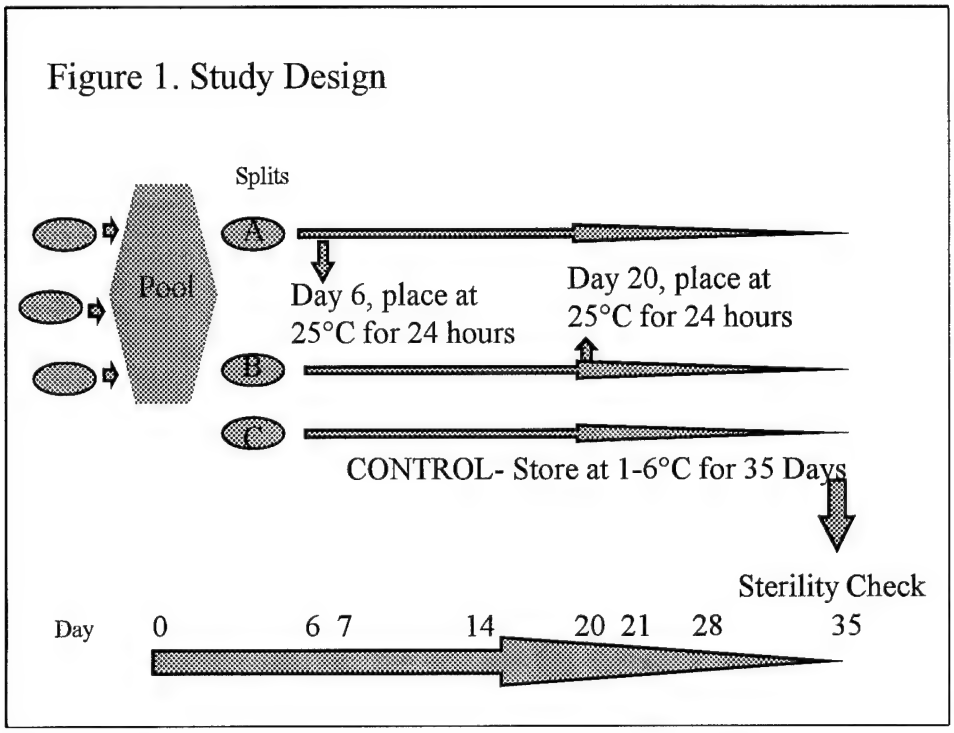
to remove was calculated using the following formulas, the first a simple ratio and the second simply uses the result of the first to determine the amount of plasma to remove.

(1) $\frac{\text{(Whole blood Weight in grams x hematocrit)}}{\text{0.65 (desired hematocrit)}} = \text{Final weight}$

(2) Whole blood Weight - Final weight = weight of plasma to remove

Pooled and Split Packed Red Blood Cells

The red blood cells were mixed in the primary bag by hand in a figure eight motion 10 times. The PRBC in each of 3 primary bags in a pool were sterile docked (Terumo sterile docker, Elkton, MD) and transferred to a 1000 mL transfer pack (Baxter Health Care Corporation, Fenwal Division, Deerfield, IL, Code 4R2032) (Figure 1). After transfer each primary bag was removed by heat seal (Hematron Dielectric sealer). The blood was mixed during pooling and prior to splitting. Fifteen mL of blood was sampled from the 1000 mL bag using a sampling site coupler (Fenwal Division, Deerfield, IL, 4C2405) for day zero testing. The 1000 mL bag was weighed; the weight was divided by three to determine the volume which should be placed in each split. The primary collection bag was removed from a CPDA-1 quadruple bag collection system (Fenwal, Baxter Health Care Corporation, Deerfield, IL) by heat seal and the 3 satellite bags were saved. The 3 satellite bags were sterile docked to the 1000 mL transfer bag and an equal volume was allowed to flow by gravity into each of the 3 satellite bags. The bags were weighed to ensure an equal volume in each unit labeled A, B, and C. The units were separated from each other and from the primary bag by heat sealing. The blood from three volunteers was pooled for two reasons. First, pooling allowed



Blood was collected from 24 volunteers and pooled in 8 groups of three; each pool having the same blood group. This figure represents one pool. Three PRBC (small ovals) were pooled (hexagon), mixed, sampled and split three ways (ovals A, B, and C). Therefore, each split of the pool had the usual PRBC unit volume of approximately 250 mL. The splits designate A were removed from 1-6°C storage at day 6 and warmed in a 25°C incubator for 24 hours, and then returned to the refrigerator; splits designated B were warmed at day 20 instead. Controls, designated C, were refrigerated continuously for 35 days. Samples were taken from all splits at day 6, 7, 14, 20, 21, 28, and 35. Sterility was checked at day 35.

maintenance of the usual, ordinary PRBC unit volume of approximately 250 mL despite frequent sampling. Therefore, this allowed us to retain the customary surface-to-volume ratios of PRBC units and more closely mimic situations encountered in clinical practice. Splitting a single donation three ways would have resulted in very low residual volumes at the end of the storage period. Second, pooling normalizes the inherent variability in red cell storage characteristics among donors. A segment, to be used in the visual examination, was made from the tubing connecting the satellite bags by heat seal and taped to the back of each unit. The blood was stripped from the remaining tubing and a temporary clamp placed on the line. The units A, B, and C were placed in refrigerated storage (1-6°C) at approximately 4 hours post phlebotomy (Nor-Lake Scientific, double door blood bank refrigerator). Blood is normally refrigerated four hours post phlebotomy because of processing time and transport from mobile sites.

Sampling

Samples were taken on day zero from the 1000 mL transfer pack (pool of three units). All other samples were taken from units A, B, and C. Sampling was performed in a biological safety cabinet (Nuaire, Plymouth, MN) to prevent bacterial contamination of the PRBC units. A sampling site coupler (Fenwal, 4C2405) was used on the 1000 mL transfer pack at day zero and on the split units A, B, and C on day 35. A sampling site coupler was inserted into an available port, and wiped with 70% isopropyl alcohol. The units were mixed in a figure 8 motion 10 times prior to sampling. A 20 mL syringe with a 16 gauge needle was used for sampling. Fifteen mL of RBC was removed from each of

the eight 1000 mL transfer bags at day zero and from each unit A, B, and C at day 35. Ten mL was removed prior to the 15 mL sampling through a new port for sterility testing on day 35. The alternative sampling method was used to sample each unit A, B, and C at days 6, 7, 14, 20, 21, and 28. The clamp was removed from the tubing; forceps were placed about two inches from the end of the tubing; and the end was cut with scissors. The forceps were released while maintaining light pressure on the blood bag, and the 15 mL of blood drained into three prelabeled test tubes. The forceps were reapplied and the tubing resealed using the heat seal. The line was stripped and reclamped.[42] This alternate sampling method was used to avoid the possible contamination of the units because of failure of the sampling site coupler septum following multiple punctures. Sampling site couplers are not meant for repeated sampling and may allow leakage of blood or air. The units were placed in refrigerated storage after sampling.

Incubation of Test Unit

On day 6, all units from each pool were sampled, units labeled A were removed from refrigerated storage and placed in a 25°C incubator for 24 hours. On day 7, at the end of the incubation, all units from each pool were sampled and placed back in refrigerated storage. On day 20 the process was repeated except, units labeled B were removed from refrigerated storage and placed in a 25°C incubator for 24 hours. On day 21 at the end of the incubation, all units in each pool were sampled and placed back in refrigerated storage.

Tests on Samples Taken:

pH

Each day of sampling a blood sample was taken either directly from a 20 mL or 1 mL syringe and analyzed with a clinical blood gas analyzer (CIBA Corning 855 Blood Gas Analyzer, Medfield, MA). The analyzer used an ion specific electrode to detect the hydrogen ion concentration.

Deproteinization

The whole blood samples were deproteinized for whole blood glucose, ATP, and lactate testing. Deproteinization was performed as follows. For each sample to be processed, 3.0 mL of RBC in a 12x75 mm test tube and 6 mL of 12% perchloric acid (PCA) (Sigma Diagnostics, St. Louis, MO catalog # P7641) were chilled on ice in a 50 mL conical polypropylene tube (Baxter, Deerfield, IL, Catalog No. C-3920-50). The RBC sample was added to the PCA drop-wise while mixing with a vortex mixer, and was left on ice for 10 to 15 minutes to precipitate. The sample was separated by centrifugation at 4°C at 2700 x g for 10 minutes (refrigerated centrifuge, Beckman Instruments, Fullerton, CA). The supernatant was decanted into a 15 mL conical tube and centrifugation repeated. Five hundred μL of the supernatant was removed and placed in a pre-labeled microtube, frozen, and stored at -80°C for lactate testing at a later time (aliquot for lactate must be removed prior to pH adjustment). While vortex mixing, 6 mL of deproteinized sample was added to 1.2 grams of $KHCO_2$ in a separate 50 mL conical tube and the pH adjusted to 8-9 as measured with pH paper (ColorpHast, EM Reagents, Catalog No. 9583). The mixture was incubated on ice for 10-15 minutes. The

deproteinized supernatant was separated from the $KHCO_2$ by centrifugation at 4°C at 2700 x g for 10 minutes. After centrifugation 500 µL of the supernatant was placed in a pre-labeled microtube, frozen, and stored at -80°C for glucose testing at a later date. The remainder of the supernatant was placed in a pre-labeled test tube and frozen at -80°C for ATP testing at a later date. [43, 44, 45]

Hematology Testing

A. Hemoglobin and Hematocrit

The hemoglobin and hematocrit were measured with a clinical hematology analyzer (Baker Hematology Series Cell Counter System 9000, Allentown, PA). The Hgb was measured directly by the cyanomethemoglobin method and the hematocrit was calculated by the instrument.

B. Spun Hematocrit (only performed on day zero to ensure a value <80%)

Two capillary tubes (1.55 mm diameter, 75 mm long, Sherwood Medical Industries, St. Louis, MO, Catalog No. 6630-00-618-0013) were filled with the RBC sample and one end was sealed with clay. The cells were packed by centrifugation in a microhematocrit centrifuge for 2.5 minutes at 13,000 x g. The hematocrit was read manually on a microhematocrit reader (International Equipment Company, Needham Hts, MA, catalog No. 2201).[46, 47, 48]

Determination of Human Erythrocyte Morphology Index

The cells were fixed by adding a 20 µL sample of RBC to 1 mL of 0.5% glutaraldehyde (Sigma Diagnostics grade II, St. Louis, MO, G-6257) in phosphate

buffered saline at room temperature. The mixture was incubated at room temperature for 10 minutes and was then filled with saline and separated via centrifugation at 2000 rpm in a serological centrifuge (Clay Adams Inc., Sero-fuge II, Cat. No. 0541) for 1 minute and the supernatant was decanted. The cells were washed with saline four more times then resuspended in saline for future testing. This scoring took long periods of time so saline was replaced weekly until the scoring occurred.

The morphology index was performed according the method of Usry, Moore, and Manalo as modified in the laboratory of Meryman.[49] The glutaraldehyde fixed cells were resuspended in saline, and two drops were added to two mL of distilled water. After mixing, one drop of this suspension was placed on a glass slide and a cover slip was applied. Two hundred cells were counted at 45x power magnification using a light microscope. Each of the 200 cells was placed in one of six categories. The categories were discocyte, type 1, 2, and 3 echinocyte, echino-spherocyte, and spherocyte. A discocyte (D)is a smooth bi-concave disc (a normal shaped RBC). A type 1 echinocyte (T1E) is an irregularly shaped RBC without bumps evident on the membrane. A type 2 echinocyte (T2E) is a crenated discoid with blunt, rounded protrusions erupting from the membrane surface. A type 3 echinocyte (T3E) is a crenated spheroid with pointed, spiky protrusion erupting from the surface of the membrane. A echinospherocyte (ES) is a crenated sphere, almost completely round which has very fine hair-like protrusions from its membrane surface. A spherocyte (S) is a smooth dense sphere, which is smaller than a discocyte and is completely round with no protrusions from its surface.[50, 51] The

following formula was used to calculate the morphologic index:

$$\frac{\#D(1) + \#T1E(0.8) + \#T2E(0.6) + \#T3E(0.4) + \#ES(0.2) + \#S(0)}{\text{Total number of cells counted}} \times 100$$

Plasma Na+ and K+

Plasma was separated from a 14 mL of the PRBC sample by centrifugation at 2700 x g for 20 minutes. Na+ and K+ were determined using ion-specific electrodes (Roche Diagnostic Systems Cobas Fara, Nutley, NJ). [52, 53]

Whole Blood Glucose

The assay is based on the enzymatic conversion of glucose by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD) as follows:

glucose + ATP ----------HK--------$>>$ glucose-6-phosphate + ADP

glucose-6-phosphate + NAD^+ --------G6PD----------$>>$ 6-phosphogluconate + NADH

NAD^+= nicotinamide adenine dinucleotide

The production of NADH is measured spectrophotometrically at 340 nm and is directly proportional to the concentration of glucose.[54, 55] The deproteinized sample was thawed and glucose analyzed on a clinical chemistry analyzer (Roche Diagnostic Systems Cobas Fara, Nutley, NJ).

ATP Determination

ATP levels were determined by the decrease in absorbance at 340 nm that results when NADH is oxidized to NAD^+ in the following reactions. The ATP test is based on the reactions described by Bucher as modified by Adams.[45]

ATP + 3-Phosphoglycerate ---------PGK------$>>$ADP + 1,3-Diphosphoglycerate

PGK = phosphoglycerate phosphokinase

1,3-Diphosphoglycerate + NADH $\xrightarrow{\text{GAPD}}$ Glyceraldehyde-3-P +NAD^+ + P

GAPD = glyceraldehyde phosphate dehydrogenase

A commercially available adenosine-5'-triphosphate kit (Sigma Diagnostics, # kit 35-B, St Louis, MO) was used. One mL of PGA buffered solution, 1.5 mL of distilled water, and 0.5 mL of deproteinized sample were added to each vial containing 0.3 mg NADH and mixed several times. The contents of the vial were decanted into a 4 mL spectrophotometer cuvette and the absorbance versus distilled water was taken at 340 nm (Beckman DU62, Beckman Instruments, Columbia, MO). Four tenths µL of GAPD/PGK was added to each cuvette, and the cuvette was inverted to mix. The cuvettes were incubated at room temperature for 10 minutes and the absorbance read at 340 in triplicate to ensure the end point was reached.[24, 56]

Lactate Determination

The lactate level was determined by the action of the enzyme lactate dehydrogenase (LDH) on lactate that reduces NAD^+ to NADH in the following reaction.

Lactate +NAD^+ $\xleftrightarrow{\text{LDH}}$ Pyruvate + NADH

This conversion was measured by a change in absorbance at 340 nm.

A commercially available quantitative lactate kit (Sigma Diagnostics, #228, St Louis, MO) was used. Nicotinamide adenine dinucleotide was prepared by adding 2.0 mL of Glycine Buffer, 4.0 mL of distilled water, and 0.1 mL of lactate dehydrogenase to each vial containing 10 mg of NAD^+. The deproteinized sample was thawed and lactate analyzed on a clinical chemistry analyzer (Roche Diagnostic Systems Cobas Fara,

Nutley, NJ).[57, 58]

Plasma/Supernatant Hemoglobin: Micro Drabkins Method

A modification of the method of Moore, Ledford, and Merydith was used for supernatant Hgb determination.[59, 60] Plasma was separated via centrifugation at 2700 xg for 10 minutes and frozen for batch testing. The analysis of plasma Hgb was based on the conventional cyanmethemoglobin method in which potassium ferricyanide oxidizes Hgb to methemoglobin and in turn is converted to cyanmethemoglobin using potassium cyanide.[61] The absorbance of cyanmethemoglobin at 540 nm is directly proportional to the Hgb in the sample.

The Hgb concentration can vary dramatically beyond the 100 mg/dL assay limit, samples were diluted in according to Table 2. The sample color is used to determine the sample volume.

Table 2. Sample and Working Drabkins Solution (WDS) Volumes

Sample Color	Sample Volume (μL)	Working Drabkins Solution Volume (μL)	Dilution Factor
Light to dark yellow	500	500	2
Light pink	200	800	5
Pink to light red	100	900	10
Dark red	50	950	20

Table 2 is used to determine the volume of the sample and working drabkins solution to be used, by observing the color of the sample. This chart is used in the determination of plasma Hgb to ensure proper sample volume and WDS volume.

The assay is also sensitive to plasma absorbance at 540 nm, therefore, a plasma blank

was also included. The amount of pooled plasma in the blank is adjusted according to Table 3. The sample volume must be determined using Table 2 before Table 3 can be used to determine the plasma volume and WDS volume for the plasma blank .

Table 3. Plasma and WDS Volumes for Plasma Blanks for CPDA-1 Units

If the Sample volume is:	Pooled Plasma volume (μL)	WDS (μL)
500 μL	400	600
200 μL	160	840
100 μL	80	920
50 μL	40	960

Pooled plasma volume and WDS volume to be used for the known sample volume for plasma Hgb determination. Plasma blanks should be used when determining plasma Hgb concentrations. The sample volume is determined by the color of the plasma being analyzed. Use table 2 to determine appropriate sample volume first.

The WDS and the sample were added to each cuvette. All cuvettes were mixed and then incubated for 15 minutes at room temperature. All specimens were read at 540 nm on the spectrophotometer (Beckman DU62, Beckman Instruments, Columbia, MO) against the plasma blank. A personal computer spread sheet software program (Microsoft Excel, V5, Redmond WA) was used to plot the standard curve and calculate the Hgb level. The plasma Hgb level was divided by the total Hgb level and multiplied by 100 to determine percent hemolysis using the formula:

(Plasma Hgb in g/dL / Total Hgb in g/dL) x 100 = Percent Hemolysis

Blood Culture (Sterility Check)

A commercially available blood culture system (Septi-Chek Blood Culture Bottle-Roche, Becton Dickinson Microbiology Systems, Cockeysville, MD) was used to determine if bacterial growth was present in the units of red blood cells on day 35 of storage. All procedures were performed in a biological safety cabinet.

Briefly the procedure used was as follows. A 10 mL syringe was used to take a 10 mL sample from the PRBC units. The top of the bottle and the sampling site coupler were cleaned with 70% isopropyl alcohol prior to sampling. The 10 mL sample was injected into the blood culture bottle with the syringe, using an aseptic method. The bottles were incubated at 35°C for 4 hours prior to addition of the slide assembly (Septi-Chek Slide, Becton Dickinson Microbiology Systems, Cockeysville, MD). The slide assembly was held in a vertical position, the bottle cap was removed and the slide and bottle were screwed together. To subculture, the system was tilted horizontally, and rotated so that the contents of the bottle coated the agar surfaces of the slide. The system was placed upright and incubated at 35°C. The slide was checked daily for bacterial growth and subcultured each day. The slide was observed for 4 days for evidence of growth.[62]

Visual examination

The PRBC units were checked each day of sampling for gross contamination. The color of the units was compared to the color of the segment taped to the back of each unit. Bacterially contaminated units might have gas bubbles or a color darker than the attached segments.[63]

Data Analysis

Single factor analysis of variance (ANOVA) was used to analyze the data. ANOVA is a method used to analyze three or more means. This was done by testing the null hypothesis that all three means are equal.[64] A one way ANOVA was used to compare the mean values of group A, group B, and group C at day 35. The one way ANOVA was also used to compare the mean values of group A and B at day 28 to the mean value of group C at day 35. This second analysis was performed to determine if the warmed units were as good as or better than the control group at day 35. The statistical functions of Microsoft Excel version 5.0c © 1985-1994 to Microsoft Corporation was used. The significance level used was 0.05. The program calculates the between-groups and within groups sum of squares, degrees of freedom, mean square, F statistic, P value, and gives the critical F value. If the P value is less than 0.05 then the means are not the same for each group.[65]

The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The assay values for each unit on each day, as well as the mean and the standard error for each group, can be found in Appendix A.

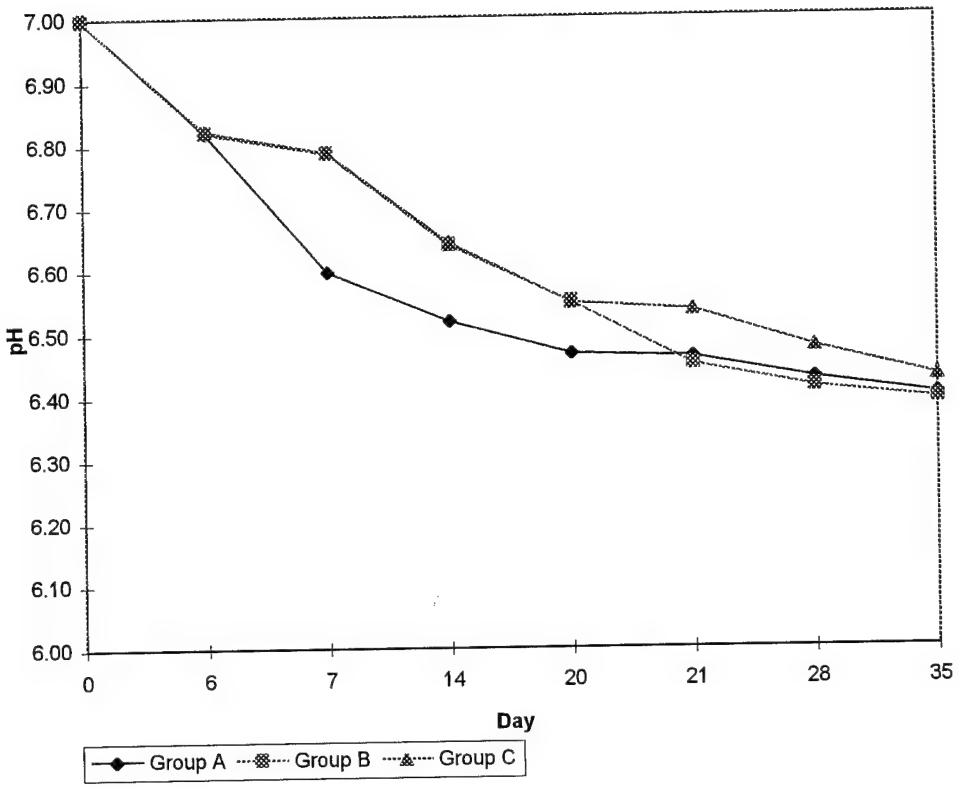
Donor Screening

All 24 PRBC units drawn had normal osmotic fragility and negative sickle cell test results. None of the donors had a history of transfusion and those with a history of pregnancy had no detectable alloantibodies. The hematocrit of all donors exceeded 38%. Of the 24 donors drawn, 3 were blood group B, 12 were blood group A, and 9 were blood group O.

pH

The pH decreased in all units tested during the 35 day storage period. The mean pH of groups A, B, and C were the same through day six, starting at 7.0 and dropping to 6.82 (Figure 2; see also Appendix A, Table A1). When group A was warmed at day six, there was a greater decrease in its mean pH (6.60) as compared to the two unwarmed groups (pH 6.79). Groups B and C had the same mean values through day 20. When Group B was warmed at day 20 the mean pH level dropped to the same level as Group A

Figure 2. Mean pH Values Over Time



The change in the pH of CPDA-1 PRBC over a normal 35 day storage period. The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The change in the pH caused by the warming of Group A can be seen at day 7 in the dramatic decrease in the pH as compared to the two unwarmed groups. This same decrease was seen in group B at day 21.

(pH 6.46). The control group had a pH higher than the warmed groups at day 21 (6.54). The mean pH of the two warmed groups was slightly lower than the control group through day 35, p value of 0.0085 (see Appendix B, Table B1). When the day 28 means of the warmed units were compared with the day 35 mean of the control group, the p value was 0.241. This indicated no statistically significant difference between the day 28 means of the warmed units and the day 35 mean of the control group.

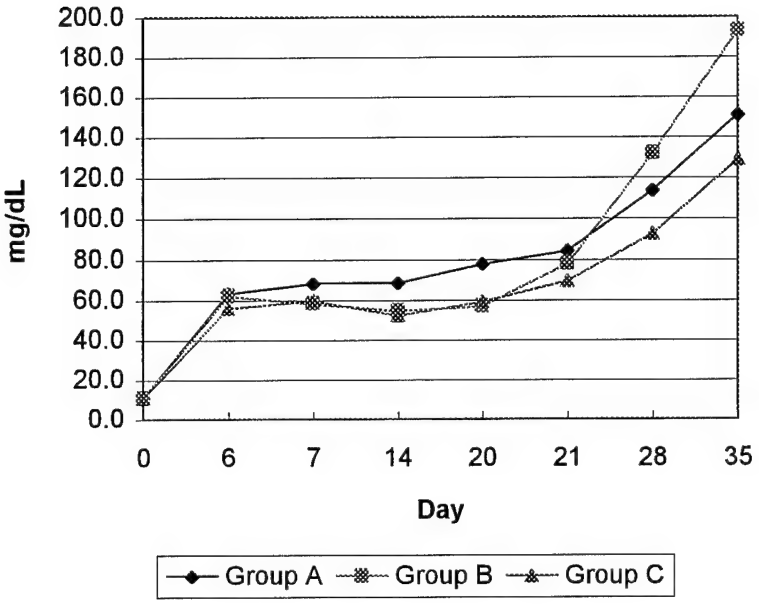
Total Hemoglobin

The total Hgb values remained constant throughout the storage period. The total Hgb values were used to calculate the percent hemolysis (see Appendix A, Table A2).

Plasma Hemoglobin

The plasma Hgb levels increased during storage as cells hemolyzed (Figure 3; see also Appendix A, Table A3). Group A had a slightly higher plasma Hgb level (68.4 mg/dL) than groups B (58.3 mg/dL) and C (60.0 mg/dL) on day seven and remained higher through day 28. The increase in the mean plasma Hgb level of group A after day 7 paralleled that of group C through day 35. Groups B and C had similar mean plasma Hgb concentrations through day 20 (57.0 and 59.1 mg/dL). When group B was warmed at day 20, the plasma Hgb concentration increased sharply to 78.0 mg/dL compared to groups A (78.0 to 84.4 mg/dL) and C (69.4 mg/dL). The mean plasma Hgb level of group B continued to rise more rapidly after warming than groups A and C. The plasma Hgb level was higher in the two warmed groups (A 151.3 mg/dL and B 193.1 mg/dL) than in the control group (129.1 mg/dL) at day 35, however, the p value was 0.051.

Figure 3. Mean Plasma Hemoglobin Levels Over Time



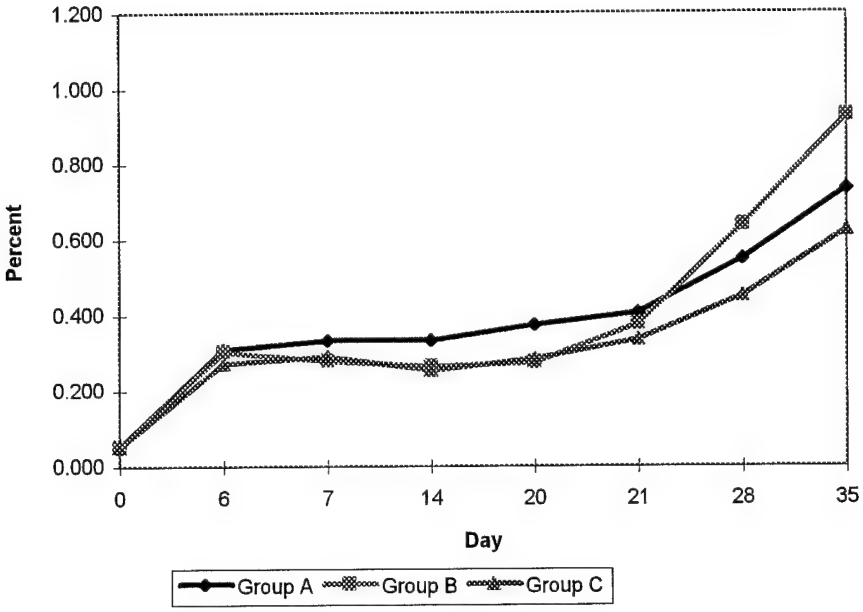
The change in the mean plasma hemoglobin levels of CPDA-1 PRBC over a normal 35 day storage period. The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The change in the plasma Hgb level caused by the warming of Group A can be seen at day 7 in the slight increase in the plasma Hgb level as compared to the two unwarmed groups. This same increase was seen in group B at day 21 with a more rapid rise in the rate of increasing plasma Hgb levels.

Although group A had higher plasma Hgb levels earlier, group B plasma Hgb levels continued to rise faster after warming having the highest mean level by day 28. When the day 28 mean plasma Hgb levels of the warmed units were compared with the day 35 mean of the control group, p was 0.62 indicating no statistically significant difference (see Appendix B, Table B2). Pool 5 had initial plasma Hgb levels five times the mean of the other units and skewed the data. Results for pool 5 for A, B, and C were removed to show a more even distribution. Pool 5 did not show abnormal results for any other assay. When pool 5 was included the day 35 mean plasma Hgb values were 169.6, 226.6, and 152.1 respectively, p was >0.05. There was not a significant difference when the mean plasma Hgb values of the warmed groups at day 28 or day 35 were compared with the day 35 mean of the control group (with or without the inclusion of pool 5).

Percent Hemolysis

The percent hemolysis was found to increases at the same rate as the plasma Hgb, since the total Hgb remained constant (Figure 4; see also Appendix A, Table A4). Only pool 5 had hemolysis greater than 1.5%. The mean of all three groups was less than 1% when pool 5 was excluded. Only the mean of group B exceeded 1% when the pool 5 values were included. The day 35 mean values with pool 5 were: group A 0.825%; group B 1.092%; and group C 0.735%. Without pool 5 the mean values at day 35 were: group A 0.734%; group B 0.927%; and group C 0.623%, p was 0.051 (see Appendix B, Table B3). When the day 28 means of the warmed units were compared with the day 35 mean of the control unit, the p value was 0.931. For percent hemolysis, there was no

Figure 4. Mean Percent Hemolysis Over Time



The change in the mean percent hemolysis of CPDA-1 PRBC over a normal 35 day storage period. The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The change in the percent hemolysis caused by the warming of Group A can be seen at day 7 in the slight increase in the percent hemolysis as compared to the two unwarmed groups. This same increase was seen in group B at day 21 with a more rapid rise in the rate of increasing percent hemolysis as compared to groups A and C.

statistically significant difference between the day 28 means of the warmed units and the day 35 mean of the control units. None of the units showed gross hemolysis.

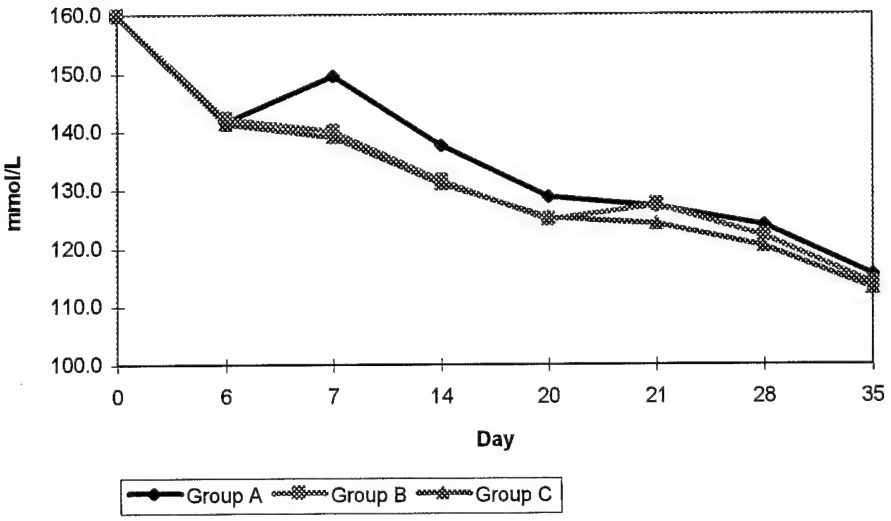
Sodium

There was a decrease in plasma Na^+ levels in all units during the 35 day storage period (Figure 5; see also Appendix A, Table A5). From day zero to day six, the plasma Na^+ levels decreased in each group, from 160 to 142 mmol/L. When group A was warmed on day six the plasma Na^+ level increased slightly to 149 mmol/L, while the levels continued to decrease in the unwarmed units, to 140 mmol/L. Group A had a slightly higher mean plasma Na^+ level through day 35. Group B and C had similar mean Na^+ levels until day 20 (124.9 and 125.4 mmol/L respectively). When group B was warmed there was an initial increase from the day before to the same level as group A at day 21 (127.4 mmol/L). The mean Na^+ levels for groups A, B, and C at day 28 were 124, 122, and 120 mmol/L respectively. At day 35 the mean Na^+ levels were 115, 114, and 113 mmol/L, p was 0.09 (see Appendix B, Table B4). When the mean Na^+ levels of the warmed groups at day 28 were compared with the mean of the control group at day 35, the p value was <0.05. The difference in the mean Na^+ levels of the warmed units at day 28 was statistically significant. The warmed units at day 28 had mean Na^+ levels that were higher than the mean control level at day 35.

Potassium

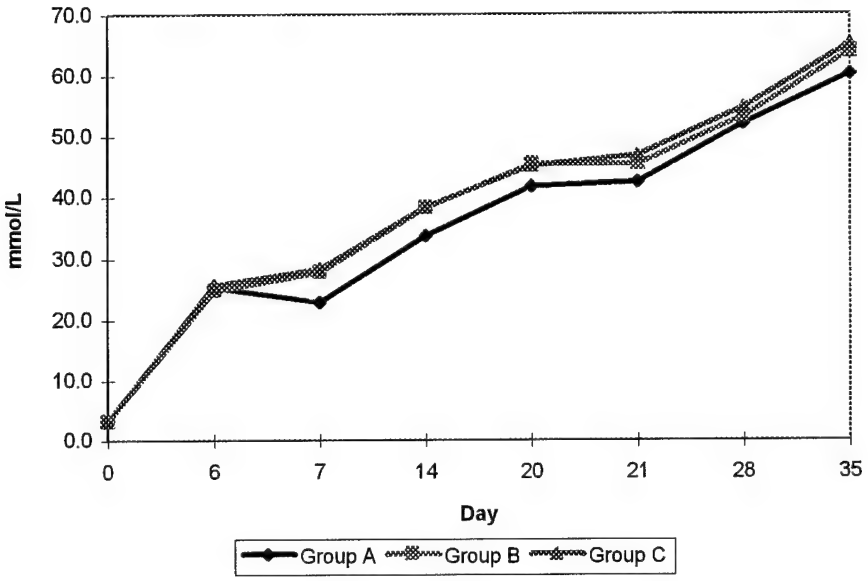
There was an increase in the K^+ levels of all units during the 35-day storage period (Figure 6; see also Appendix A, Table A6). The mean plasma K^+ level increased

Figure 5. Mean Na^+ Levels Over Time



The change in the mean Na^+ levels of CPDA-1 PRBC over a normal 35 day storage period. The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The change in the mean Na^+ level caused by the warming of Group A can be seen at day 7 in the dramatic increase in the mean Na^+ level as compared to the two unwarmed groups, which continue to decrease. A some what less dramatic increase was seen in group B at day 21.

Figure 6. Mean K^+ Levels Over Time



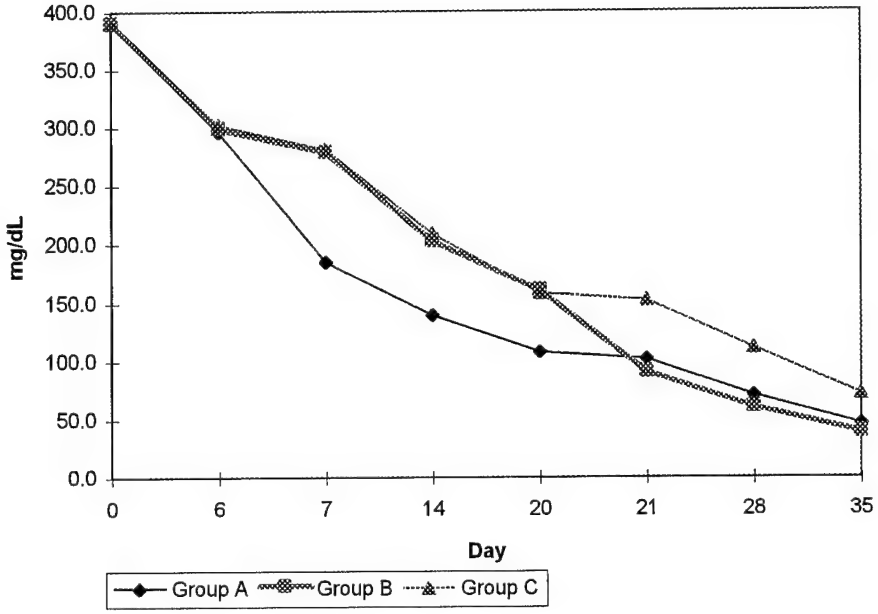
The change in the mean K^+ levels of CPDA-1 PRBC over a normal 35 day storage period. The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The change in the mean K^+ level caused by the warming of Group A can be seen at day 7 in the slight decrease in the mean K^+ level as compared to the two unwarmed groups, which continue to increase. A some what less dramatic decrease was seen in group B at day 21.

from 3.3 to 25 mmol/L in each group from day zero to day six. When group A was warmed at day six the mean plasma K^+ level decreased slightly to 23 mmol/L, while the levels continued to increase, to 28 mmol/L in the unwarmed groups. The K^+ continued to show a parallel increase in all three groups. Groups B and C had similar mean K^+ levels through day 20 (45.5 and 45.2 mmol/L respectively). When group B was warmed on day 20 the mean plasma K^+ level remained the same, while the levels in group A (41.9 to 42.6 mmol/L) and C (45.2 to 46.9 mmol/L) increased. The mean plasma K^+ level for A, B, and C at day 35 were 60.3, 63.9, and 65.2 mmol/L respectively. When the mean K^+ levels of the warmed units at day 28 and day 35 were compared with the mean of the control units at day 35, the p values were <0.05 (see Appendix B, Table B5). There was a statistically significant difference when the day 28 and day 35 mean values of the warmed groups were compared with the day 35 mean of the control group. The day 28 mean K^+ levels of the warmed groups A (52.2 mmol/L) and B (53.3 mmol/L) were lower than the mean of the day 35 control group which was 65.2 mmol/L.

Glucose

There was a decrease in the glucose level in all units tested over the 35 day storage period (Figure 7; see also Appendix A, Table A7). All groups showed similar decreases in their mean glucose values from day zero to day six, from approximately 390 to 298 mg/dL. When group A was warmed at day 6, the day seven mean (185 mg/dL) was only 65% of the group C mean (281 mg/dL). After the initial increase in the rate of glucose consumption, the group A mean glucose continued to be about 63-68% of the

Figure 7. Mean Glucose Levels Over Time



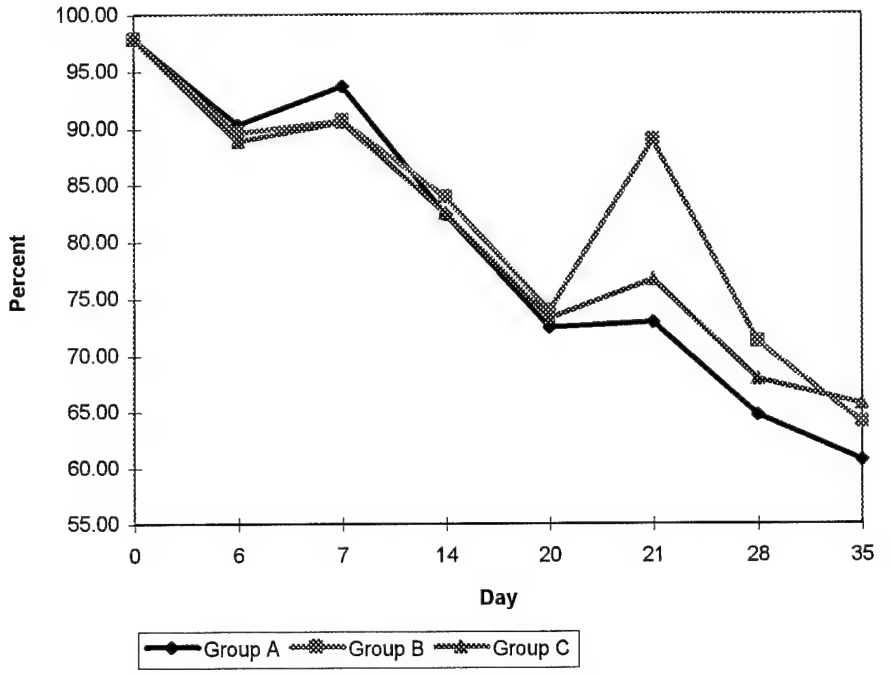
The change in the mean glucose levels of CPDA-1 PRBC over a normal 35 day storage period. The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The change in the mean glucose level caused by the warming of Group A can be seen at day 7 in the dramatic decrease in the mean glucose level as compared to the two unwarmed groups, which continue to decrease at a much lower rate. The same dramatic decrease was seen in group B at day 21. None of the units had complete depletion of glucose through day 35.

group C mean through day 35. Group B and C had similar mean glucose levels through day 20 (160 and 158 mg/dL respectively). When group B was warmed on day 20, there was an increase in glucose consumption. The group B mean glucose level (91 mg/dL) was about 60% of the group C mean (152 mg/dL) on day 21. On day 28 and 35 the group B mean glucose level was about 55% of the group C mean. The group A (46 mg/dL) and group B (39 mg/dL) mean glucose levels at day 35 were significantly lower than group C (72 mg/dL), p was .00067 (see Appendix B, Table B6). The day 28 mean glucose values for group A (mean 71.5 mg/dL) and B (mean 61.0 mg/dL) were similar to the day 35 mean glucose value of group C (mean 72.2 mg/dL), p was 0.281. Even at day 35 when the glucose levels in the warmed units were almost half that of the control units there were still detectable glucose levels in all of the units tested.

Red Blood Cell Morphology Score

RBC morphology scores decreased from day zero to day 35 in all units (Figure 8; see also Appendix A, Table A8). The mean RBC morphology score for each group decreased at the same rate from day zero to day six, from 98 % to 89%. The mean value of group A increased after warming, and group B and C remained the same from day 6 to day 7. By day 14 all three groups had similar mean morphology scores, 83%, with group A slightly lower. On day 20 all three groups had similar mean RBC morphology score, 73 %, with group A only slightly lower. After warming group B, there was a 15% increase in the mean RBC morphology score from day 20 to day 21. Groups A and C mean RBC morphology scores remained the same from day 20 to 21. By day 28 Group B

Figure 8. Mean RBC Morphology Scores Over Time



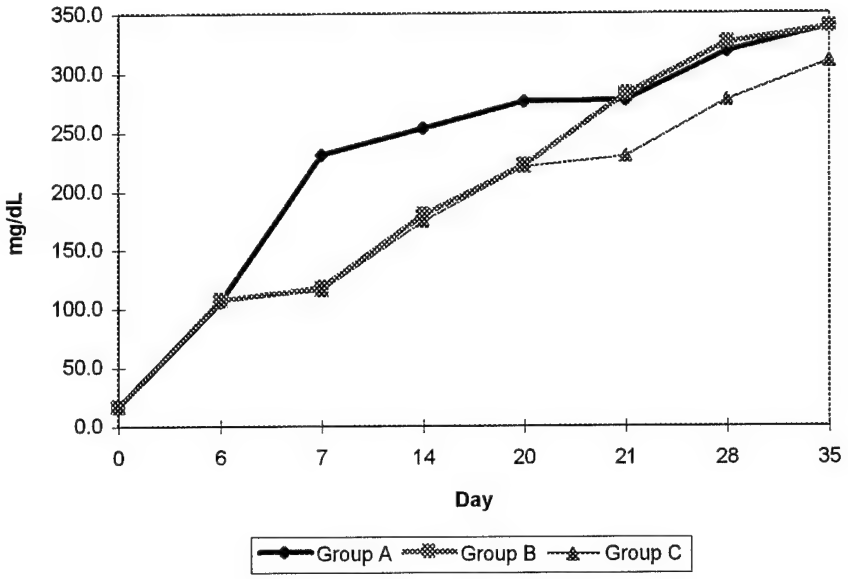
The change in the mean RBC morphology scores of CPDA-1 PRBC over a normal 35 day storage period. The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The change in the mean RBC morphology score caused by the warming of Group A can be seen at day 7 in the slight increase in the score as compared to the two unwarmed groups. A more dramatic increase in the RBC morphology score was seen in group B at day 21.

had the highest score (71%) but only 4% higher than group C (67%), and group A had the lowest (64%). By day 35 the control group had the highest score (65%), then group B (64%), and the lowest was group A (60%). On day 35 there was less than 5% difference among the three groups, p was 0.248 (see Appendix B, Table B7). The warmed units at day 28 had mean RBC morphology score as good as or better than the mean score of the control group at day 35 (p value was 0.033). The mean value of group A, at day 28, was 64%, which is similar to the control at day 35 (65%), group B's mean score was 71% at day 28.

Lactate

The mean lactate levels increased in all units during storage (Figure 9; see also Appendix A, Table A9). The mean lactate level of each group increased at the same rate from day zero to day six, from 17 to 106 mg/dL. When group A was warmed at day six, the mean lactate value doubled between prewarming and postwarming (from 105 to 231 mg/dL). Group B (117 mg/dL) and group C (115 mg/dL) increased only slightly from day six to day seven. The initial increase in the rate of lactate production in group A did not continue. The mean lactate level in group A continued to increase at a more slowly than group B or C, but had a higher mean level through day 20. When group B was warmed on day 20, there was a 21% increase (from 222 to 282 mg/dL) in its mean lactate level compared to a 3% increase in the group A and C mean levels. Group B had the highest mean lactate level through day 28 (325 mg/dL). On day 35 group A and B had the same mean lactate level, 339 mg/dL, approximately 9% higher than group C, 309

Figure 9. Mean Lactate Levels Over Time



The change in the mean lactate levels of CPDA-1 PRBC over a normal 35 day storage period. The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The change in the mean lactate level caused by the warming of Group A can be seen at day 7 in the dramatic increase in the mean lactate level as compared to the two unwarmed groups, which continue to increase at a much lower rate. A some what less dramatic increase was seen in group B at day 21.

mg/dL, p was 0.005 (see Appendix B, Table B8). When the mean lactate levels of the warmed units at day 28 were compared with the mean lactate level of the control at day 35, p was 0.276. As the glucose levels decreased sharply at day seven in group A and at day 21 in group B, the lactate levels increased sharply.

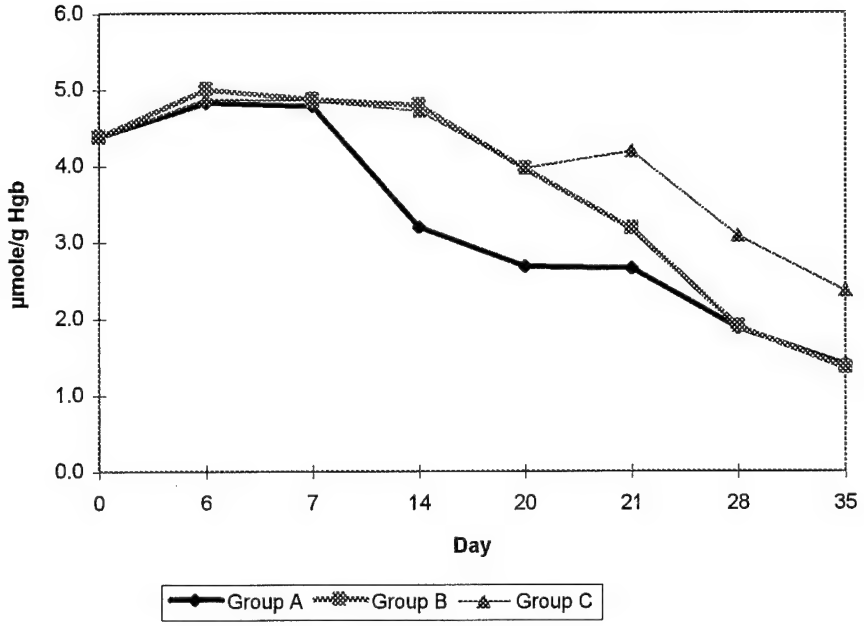
ATP

The mean ATP level of all three groups initially increased from day zero to day six (Figure 10; see also Appendix A, Table A10). There was no difference between day six and day seven values in any of the three groups. By day 14 the mean ATP level of group A had decreased to 3.2 µmole/g Hgb while group B and C remained at 4.8 µmole/g Hgb. From day 14 to day 20, mean ATP levels decreased in all groups, groups B and C had the same mean ATP levels through day 20 (4.0 µmole/g Hgb) and group A was 2.7 µmole/g Hgb. When group B was warmed at day 20 its mean ATP level decreased to 3.2 µmole/g Hgb, while A and C did not decrease. By day 28 groups A and B had the same mean ATP values, 1.9 µmole/g Hgb, and group C's mean level continued to be higher, 3.1 µmole/g Hgb. The mean ATP level of the warmed groups was 1.4 µmole/g Hgb, and the control group was 2.4 µmole/g Hgb at day 35. The mean ATP level of the control group at day 35 was significantly higher than the means of the warmed groups at day 28 and day 35, p was <0.05 (see Appendix B, Table B9).

Visual Examination

The visual examination of the PRBC showed no abnormalities in any of the units during the 35 day storage period, they appeared normal. There were no gas bubbles,

Figure 10. Mean ATP Levels Over Time



The change in the mean ATP levels of CPDA-1 PRBC over a normal 35 day storage period. The CPDA-1 PRBC units were grouped by treatment. All units were stored at 1-6°C for 35 days, with the following exceptions: the units in group A were warmed to 25°C for 24 hours at day six; the units in group B were warmed to 25°C for 24 hours at day 20. The units in group C were stored at 1-6°C for 35 days and served as the control group. Each numbered pool 1-8 had a unit in each of the groups which were the same at day zero. The effect on the mean ATP level caused by the warming of Group A at day 6 was not seen until day 14, when group A had a dramatically lower ATP level as compared to the two unwarmed groups, which did not change. When group B was warmed at day 20 the effect on the ATP level was immediately seen, in the decrease in ATP as compared to the control group at day 21.The control group mean ATP level was approximately 40% higher at day 28 and 35 as compared to the two warmed groups.

discoloration, or darkening of the unit which would indicate bacterial contamination.

Blood Cultures

The blood cultures for all units tested on day 35 and examined for 4 days thereafter showed no bacterial growth.

It is common practice to discard PRBC which reach temperatures exceeding 10°C.[2] The present project was initiated to provide data to examine the basis of this practice. If PRBC units intentionally left at 25°C for 24 hours maintain viability, then PRBC units left unrefrigerated for shorter periods of time may be used for transfusion. Blood bank medical directors may use these data to justify saving some units exposed to temperatures exceeding 10°C. Warming the units to 25°C for 24 hours was designed to simulate a worst case scenario. Experiments were designed to determine to what degree this caused acceleration of the red cell lesion, and if warming early or later during the storage period produced different results. Warming PRBC units to 25°C for 24 hours decreases shelf life by approximately one week.

An ANOVA was performed for each analyte tested. The day 35 mean of each of the three groups were compared to determine whether the means of the warmed groups were the same as the mean of the unwarmed control group. The day 28 means of the two warmed groups were also compared with the day 35 mean of the control group to determine if the warmed units had mean values as good as the control mean. If the warmed units at day 28 had mean values as good as or better than the day 35 mean values of the control group then they can be considered acceptable for transfusion up to day 28 of storage. The warmed units at day 28 were as good as or better than the control units at day 35 except for ATP measurements.

Glucose is the substrate that RBC use in the glycolytic pathway to produce ATP. The end product of glycolysis in the RBC is lactate which lowers the pH.[56] On day 35 all

group mean glucose levels were within the day 35 CPDA-1 range (74.79±55.96 mg/dL) specified by Zuck et al., and within or above the range (16±31 mg/dL) specified by Valeri et al.[34, 66] There is not a consensus in the literature for glucose levels in CPDA-1 PRBC units. Hematocrit seemed to play some role; those units with higher hematocrit having a lower glucose level with the removal of the plasma. A PRBC unit with a lesser hematocrit retains more of the preservative solution which provides the glucose. Since the present study had hematocrit values near 65%, the mean glucose levels were greater than those reported by Valeri et al, with hematocrits closer to 80%.[66] Red cells require glucose to produce ATP; if glucose is available RBC metabolism can continue. If glucose is not depleted during storage and metabolism continues the RBC gain sufficient glucose to increase metabolism upon transfusion. There was glucose present in all units; therefore, the cells had a substrate for metabolism even when they used a significant amount during the 24 hour warming period. Warming the units to 25°C for 24 hours had a significant effect on the rate of consumption of glucose in the PRBC units. This was expected since refrigeration of PRBC units slows metabolism and warming the units increases the metabolism of RBC.

Even though the mean ATP levels were low at day 28 in the warmed groups, they still were high enough for the PRBC to be used in transfusion according to current guidelines. At day 28 groups A and B had mean ATP levels of 1.9 µmole/g Hgb, which are within the range (1.9±.52 µmole/g Hgb) for day 35 CPDA-1 PRBC units specified by Zuck et al.[34] Valeri et al. also found a similar range for ATP levels in 35 day CPDA-1

PRBC .[66] The warmed units at day 28 had 43% of their original ATP remaining which was within the range (45.7±12.3% of initial ATP level) specified by Simon.[67] Group C values were within the range specified by Zuck et al at day 35.[34] The warmed units lost one week of storage time, because of the loss of ATP incurred during the 24 hour warming period. Cooling the PRBC units slows the metabolism of the RBC, therefore warming the PRBC increases RBC metabolism and the use of ATP.[2, 14] At day 35 the ATP levels of the warmed units had dropped below the critical 1.5 μmole/g Hgb required for cell life, and therefore would not be acceptable for transfusion. Red blood cells do not survive with less than 1.5 μmole ATP/g Hgb. Above this level the RBC can be regenerated post-transfusion but below this level there would not be >70% survival of transfused RBC 24 hours post-transfusion.[56] The 24 hour storage at 25°C had the same impact as 1 week of refrigerated storage on the ATP levels of the PRBC.

Lactate, the end product of red cell metabolism, will show an increase any time that the metabolism of the red cells is increased. Normal storage of blood at refrigerated temperatures slows metabolism and, therefore, the production of lactate. When the PRBC were warmed there was a more dramatic rise in lactate levels than is normal for a PRBC unit. However, since lactate is buffered by proteins and Hgb in the PRBC, the great increase in lactate caused only a slight decrease in the pH.[68] The mean pH values of the 28 day old warmed units were within the range (6.46±0.05) specified for 35 day CPDA-1 PRBC units by Zuck et al.[34] The mean of the control group was within the range at day 28 and 35. Lactic acid normally accumulates during storage and will be detoxified in the

liver. This accumulation of lactic acid may cause problems in patients with hepatorenal deficiency and physicians normally use fresh PRBC or whole blood for these patients.[69] There was not a significant difference between the day 28 mean lactate levels of the warmed units and that of the day 35 control mean. Therefore, warming should not cause any abnormal effects from the increase in lactate if the PRBC is transfused by day 28.

On day 35 all group mean Na^+ levels were within the day 35 values (112.84±15.40 mmol/L) for CPDA-1 specified by Zuck at el.[34] The increase in Na^+ and the decrease in K^+ caused by warming were not sufficient to be of clinical use, but show reactivation of the Na^+/K^+ pump with warming. Because the trend of decreasing plasma Na^+ and increasing plasma K^+ is reversed temporarily after warming, the Na^+/K^+ pump might have been reactivated. Although the cells regain some ability to generate a normal Na^+/K^+ balance it is not enough to be beneficial to the patient compared to an unwarmed PRBC unit.

The reactivation of the Na^+/K^+ pump may be one of the factors responsible for the rise in the RBC morphology score. The RBC regained a more normal shape after being warmed to 25°C. Transfusion of blood into a warm body may cause this same effect. During storage RBC are ATP depleted, which leads to accumulation of excess intracellular Ca^{++}, and Na^+, followed by K^+ and water loss, resulting in dehydrated, rigid RBC which are sequestered by the spleen.[14] Low ATP levels in PRBC first leads to reversible disulfide crosslinking, and then, finally, irreversible polymer formation within the membrane skeleton.[21] Once these irreversible changes occur the RBC will not be

useful for transfusion. Red blood cells must retain the flexibility given by their discocyte shape in order to function and move through small blood vessels. The reticuloendothelial system is designed to remove cells that are not shaped correctly.[18] Another factor that may contribute to the increased RBC morphology score would be the loss of some of the spherocytes through hemolysis, since there was a small increase in hemolysis after warming. These spherocytic cells would be lost to the reticuloendothelial system once transfused.[21] It might be interesting to determine how long a unit must be placed at room temperature before there is an increase in RBC morphology score. If this were attempted it would also be necessary to do in-vivo survival studies to determine if RBC with a higher morphologic score actually survive better.

The plasma K^+ level for all of the groups at day 35 were within the range (87.59±22.6 mmol/L) specified by Zuck et al. for 35 day old CPDA-1 PRBC.[34] Although pool 5 showed a plasma Hgb much greater than the other pools there was no effect on its plasma K^+ level. It is thought that when cells hemolyze they will release enough K^+ to cause a significant increase in the plasma levels. The method used to determine plasma Hgb levels was designed to lyse microvesicles. These microvesicles shed by the RBC contain Hgb. Much of the plasma Hgb is found in microvesicles.[33] If the cells in pool 5 lost many microvesicles, the cells could lose Hgb without releasing the intracellular K^+. Cells maintain a Na^+ and K^+ gradient across the cell membrane by primary active transport, which utilizes the direct hydrolysis of ATP for the movement of ions. Potassium is pumped into the cell; Na^+ is pumped out.[70] During storage RBC metabolism

is slowed and the Na^+/K^+ pumps cannot maintain a normal balance leading to the increase in plasma K^+ and decrease in plasma Na^+. Without ATP the Na^+/K^+ pump will not function.[14] A patient may become hyperkalemic from the intracellular loss of K^+ from RBC into the plasma, during storage. A patient can become hypokalemic if transfused with intracellular K^+ depleted RBC components, such as washed PRBC. Hyperkalemia or hypokalemia may cause cardiac arrhythmias and seizures.[18] Warming the units to 25°C did not cause the K^+ levels to increase, and it did not cause conditions that would cause hypokalemia.

Total Hgb values are not affected by warming the units. Hemoglobin is a stable protein and would not be affected by storage at 1-6°C or 25°C. Improper sampling of inadequately mixed PRBC could lead to a significant change in total Hgb values.[71] Knowing that improper mixing of PRBC units could influence whole blood test results, the Hgb concentration was evaluated to ensure that mixing was not a factor in the study. Total Hgb was monitored to check for adequate consistent mixing.

All day 35 plasma Hgb values (including pool 5) were below the values found by Zuck at el. whose mean value was 524.59 mg/dL.[34] Lesser mean plasma Hgb levels indicate that there are more intact cells in the PRBC to transfuse. Warming the units may lead to lysis of the cells if the temperature rises above the normal body temperature of 37.5°C. Since groups A and B were only warmed to 25°C a significant increase in plasma Hgb was not expected and was not found.

Gross hemolysis may cause a significant increase in the plasma potassium level.

The mean potassium level of the warmed groups was lower than that of the control. There was not gross hemolysis detected in any of the units. The mean plasma Hgb of the warmed groups at day 28 was equal to the mean plasma Hgb level of the control group at day 35. This would indicate that a unit warmed to 25°C for 24 hours during a 35 day storage time would have a plasma Hgb low enough at day 28 to allow transfusion.

The warmed units all had acceptable ATP, glucose, K^+, Na^+, morphology, pH, lactate, and hemolysis levels at day 28. The warming had transient beneficial effects on RBC morphology scores, and Na^+ and K^+ levels. The warming caused an acceleration of ATP depletion, and glucose utilization which gave rise to these temporary beneficial effects. The cells appeared better after warming but lost storage time. The cell membrane would be more deformable at temperatures closer to body temperature. This deformability allows more normal survival of the RBC. If in vivo survival studies indicated that the cells survived better after warming then perhaps some day physicians may allow PRBC units to warm to room temperature prior to transfusion. This may already occur to some extent since PRBC units are transfused within four hours after leaving the blood bank. Also, to rejuvenate PRBC prior to freezing, the PRBC are warmed with an additive solution. Red blood cells can be rejuvenated up to three days past their allowable storage and have increased levels of 2,3-DPG and ATP.[2] Warming the RBC during the rejuvenation process plays a key role, allowing cells to use the additives more readily.

Still, one of the primary concerns of transfusing a warmed PRBC unit is bacterial

contamination. Because of this concern, before a unit of PRBC is issued for transfusion technologists perform a visual check for gross contamination. The appearance of the PRBC units was satisfactory through day 35 of the present study, but units that appear normal can still be contaminated. Experimental methods could have introduced bacterial contamination at several points during manipulation of the PRBC units, i.e., pooling and additional nine samplings. PRBC units are normally transfused within 24 hours after the hermetic seal has been broken if the unit is stored at 1-6°C or 4 hours if stored at room temperature.[2] There were increased chances for bacterial contamination during the present study, yet none of the 24 units was contaminated with bacteria.

A PRBC unit warmed to 25°C for 24 hours will not retain the storage life of a PRBC unit that has been stored at 1-6°C. The storage lesion is accelerated to the point that there is not adequate ATP at day 35, warmed groups had mean ATP levels of 1.4 μmole/g Hgb which is below the 1.5 μmole/g Hgb needed to maintain cell life. However, the warmed groups had adequate mean ATP levels at day 28 (1.9 μmole/g Hgb) to be transfusable. It appears that a day of 25°C storage of CPDA-1 PRBC accelerates aging equivalent to a week of conventional storage at 1-6°C. It did not appear to matter whether the PRBC are warmed at day 6 or day 20. There were no in-vitro effects that would preclude transfusion, and no bacterial contamination of PRBC stored at 25°C for 24 hours.

Medical directors may find this information useful in logistically difficult circumstances and they may save rare or expensive units of blood. Blood is difficult to

obtain and a valuable resource. This is true of common blood types and even more so for rare types. The common practice of discarding blood that reaches 10°C seems to be conservative in light of the data presented here. CPDA-1 PRBC units appear to be able to withstand a full day at 25°C with only a 20% decrease in their shelf life.

1. American Association of Blood Banks, Standards Committee. Standards for blood banks and transfusion services. 16th ed. Bethesda, MD: American Association of Blood Banks, 1994: 18-19.

2. American Association of Blood Banks, Technical Manual Committee. Technical manual. 11th ed. Bethesda, MD: American Association of Blood Banks, 1993:51-73.

3. Food and Drug Administration. Code of federal regulations: food and drugs 21: Parts 600 to 799. Washington, DC: U.S. Government Printing Office, 1995:116 and 118.

4. American Association of Blood Banks, Technical Manual Committee. Technical Manual. 11th ed. Bethesda, MD: American Association of Blood Banks, 1993:718.

5. Lewisohn R. A new and greatly simplified method of blood transfusion: a preliminary report. M Rec 1915; 87: 141-142.

6. Lewisohn R. The development of the technique of blood transfusion since 1907: with special reference to contributions by members of the staff of the Mount Sinai Hospital. J Mt Sinai Hosp 1944;10: 605-622.

7. Meeting of the Subcommittee on Blood Substitutes, Division of Medical Sciences. Minutes. NRC: 30 November 1940.

8. Kendrick DB. Blood program in world war II. Washington DC: Medical Department, United States Army, 1989:217-232.

9. Conference on Transfusion Equipment and Procedure, Division of Medical Science. Minutes. NRC:25 August 1942.

10. Conference on Preserved Blood, Division of Medical Sciences. Minutes. NRC:25 May 1943.

11. Conference on Blood Preservation, Division of Medical Sciences. Minutes. NRC:19 January 1945.

12. Conference on Blood Preservation, Division of Medical Sciences. Minutes. NRC:8 February 1945.

13. Elliott J. to Chief, Surgical Consultants Division, Office of the Surgeon General, Memorandum: Transportation of blood from the U.S. to the ETO blood bank in Paris. 1 February 1945.

14. Harmening DM, ed. Modern blood banking and transfusion practices. 3rd ed. Philadelphia: F.A. Davis Company, 1994:1-25.

15. Devlin TM, ed. Textbook of biochemistry with clinical correlations. 3rd ed. New York: Wiley-Liss, Inc., 1992:293.

16. Moroff G, Holme S, Heaton WAL, Kevy S, Jacobson M, and Popovsky M. Effect of an 8 hour holding period on in vivo and in vitro properties of red cells and factor VIII content of plasma after collection in a red cell additive system. Transfusion 1990;30:828-832.

17. Moore GL, Ledford ME, and Bolin RB. The effects of an eight hour hold prior to component preparation on red cells drawn in CPDA-1. Transfusion 1980:Sept.-Oct:644.

18. Harmening DM, ed. Modern blood banking and transfusion practices. 3rd ed. Philadelphia: F.A. Davis Company, 1994:351-374.

19. Hamill TR, Hamill SG, and Busch MP. Effects of room-temperature exposure on bacterial growth in stored red cells. Transfusion 1990;30:302-305.

20. American Association of Blood Banks, Technical Manual Committee. Technical Manual. 11th ed. Bethesda, MD: American Association of Blood Banks, 1993:1-28.

21. Wolfe LC. The membrane and the lesions of storage in preserved red cells. Transfusion 1985;25:185-203.

22. Pietersz RNI, Dekker WJA, and Reesink HW. Sterility of blood products derived from whole blood stored at ambient temperature for 16 to 24 hours before separation (abstract). Transfusion 1991;31(Suppl):62S, S232.

23. Mazor D,Dvilansky A, and Meyerstein N. Prolonged storage of red cells: the effect of pH, adenine and phosphate. Vox Sang 1994;66:264-269.

24. Anderson KC, and Ness PM, ed. Scientific basis of transfusion medicine: implications for clinical practice. Philadelphia: W.B. Saunders Company, 1994: Ch.10.

25. Greenwalt TJ, Sostok CZ, and Dumaswala UJ. Studies in red blood cell preservation: 1. effect of the other formed elements. Vox Sang 1990;58:85-89.

26. Moore GL. Long-term storage and preservation of red blood cells. Biotechnology 1991;19:31-46.

27. Vora S, West C, and Beutler E. The effect of additives on red cell 2,3-diphosphoglycerate levels in CPDA preservatives. Transfusion 1989;29:226-229.

28. Heaton A, Keegan T, and Holme S. In vivo regeneration of red cell 2,3-diphosphoglycerate following transfusion of DPG-depleted AS-1, AS-3 and CPDA-1 red cells. Br J Haematol 1989;71:131-136.

29. Greenwalt TJ, Sostok CZ, and Dumaswala UJ. Studies in red blood cell preservation: 2. comparison of vesicle formation, morphology, and membrane lipids during storage in AS-1 and CPDA-1. Vox Sang 1990;58: 90-93.

30. Dumaswala UJ, Petrosky TL, and Greenwalt TJ. Studies in red blood cell preservation: 6. red cell membrane remodeling during rejuvenation. Vox Sang 1992;63:12-15.

31. Greenwalt TJ, Bryan DJ, and Dumaswala UJ. Erythrocyte membrane vesiculation and changes in membrane composition during storage in citrate-phosphate-dextrose-adenine-l. Vox Sang 1984;47:261-270.

32. Meryman HT, Hornblower ML-S, and Syring RL. Prolonged storage of red cells at 4°C. Transfusion 1986;26:501-505.

33. Greenwalt TJ, McGuinness CG, and Dumaswala UJ. Studies in red blood cell preservation: 4. plasma vesicle hemoglobin exceeds free hemoglobin. Vox Sang 1991;61:14-17.

34. Zuck TF, Bensinger TA, Peck CC, Chillar RK, Beutler E, Button LN, McCurdy PR, Josephson AM, and Greenwalt TJ. The in vivo survival of red blood cells stored in modified CPD with adenine: report of a multi-institutional cooperative effort. Transfusion 1977;17:374-382.

35. Moroff G, Holme S, Keegan T, and Heaton A. Storage of ADSOL-Preserved red cells at 2.5 and 5.5°C: comparable retention of in vitro properties. Vox Sang 1990;59:136-139.

36. Greenwalt TJ, McGuiness CG, Dumaswala UJ, and Carter HW. Studies in red blood cell preservation: 3. a phosphate-ammonium-adenine additive solution. Vox Sang 1990;58:94-99.

37. Dacie JV, and Vaughan JM. The fragility of the red blood cells: Its measurement and significance. J Path Bact 1938;46:340.

38. Becton-Dickinson. Unopette Test 5830 Product Insert. Rutherford, NJ.

39. Harmening DM. Clinical Hematology and Fundamentals of Hemostasis. 2nd ed. Philadelphia: F.A. Davis Company, 1992:540-554.

40. Columbia Diagnostics, Inc. Sickle Cell Reagent Test Kit, Package Insert. Springfield, VA. July 1993.

41. American Association of Blood Banks, Technical Manual Committee. Technical Manual. 11th ed. Bethesda, MD: American Association of Blood Banks, 1993:721-722.

42. Moore G. Personal communication with Lloyd E. Lippert. Sampling Method. 12 February 1996.

43. American Red Cross Blood services - Tidewater Research. Red blood cell sample processing for in vitro testing. Norfolk, VA. 9 October 1990.

44. Sigma chemical Company. Lactate: quantitative, enzymatic determination of lactate in whole blood at 340 nm. Sigma Diagnostics Procedure No. 826-UV. St. Louis, MO. October 1990.

45. Sigma Chemical Company. Adenosine-5'-triphosphate (ATP): quantitative, enzymatic determination in blood at 340 nm. Sigma Diagnostics Procedure No. 366-UV. St. Louis, Mo. March 1989.

46. Harmening DM. Clinical hematology and fundamentals of hemostasis. 2nd ed. Philadelphia: F.A. Davis Company, 1992:56-57.

47. American Association of Blood Banks, Standards Committee. Standards for blood banks and transfusion services. 16th ed. Bethesda, MD: American Association of Blood Banks, 1994:3 and 37.

48. Food and Drug Administration. Code of federal regulations: food and drugs 21: Part 640. Washington DC: U.S. Government Printing Office, 1995:3.3.

49. Mesbah-Karimi N, of Dr. H. Meryman's Group. Personal Communication. Red blood cell morphology scoring. March-May 96.

50. Usury RT, Moore GL, and Manalo FW. Morphology of stored rejuvenated human erythrocytes. Vox Sang 1975;28:176-183.

51. American Red Cross Research, Mid-Atlantic Region. Morphology of human erythrocytes - protocol. Norfolk, VA. 11 January 1994.

52. Roche Diagnostic Systems. Roche standards for the COBAS ISE module product insert. Nutley, NJ. April 1989.

53. Tietz NW. Fundamentals of clinical chemistry. 2nd ed. Philadelphia: W.B. Saunders Company, 1982:873-884.

54. Roche Diagnostic systems. Roche Reagents for Glucose Product Insert. Nutley, NJ. March 1993.

55. Tietz NW. Fundamentals of clinical chemistry. 2nd ed. Philadelphia: W.B. Saunders Company, 1982:240-244 and 248-249.

56. American Red Cross Blood services - Tidewater Research. Spectrophotometric determination of red blood cell ATP. Norfolk, VA. 7 August 1989.

57. Sigma Chemical Company. Lactate: quantitative, enzymatic determination in blood at 340 nm. Sigma Diagnostics Procedure No. 826-UV. St. Louis, Mo. October 1990.

58. American Red Cross Blood services - Tidewater Research. Lactic acid in whole blood, platelet suspensions, and plasma. Norfolk, VA. 7 August 1989.

59. Moore GL, Ledford ME, an Merydith A. A micromodification of the drabkin hemoglobin assay for measuring plasma hemoglobin in the range of 5 to 2000 mg/dL. Biochem Med 1981;26:167-173.

60. Mechling ML. Plasma/supernatant hemoglobin - micro drabkin method. Standard Operating Procedure B411-218. Walter Reed Army Institute of Research, Blood Storage Laboratory. Rockville, MD. January 1996.

61. American Red Cross Blood services - Tidewater Research. Determination of supernatant hemoglobin (cyanmethemoglobin method). Norfolk, VA. 25 May 1993.

62. Becton Dickinson Microbiology Systems. Procedure for the culture of microorganisms when used with the BBL septi-chek blood culture bottle. MD. 30 October 1995.

63. Brecher ME. Bacterial contamination of blood products. Fourteenth Annual Symposium: Immunohematology and blood transfusion. 12 September 1995.

64. Shott S. Statistics for Health Professionals. Philadelphia: W. B. Saunders Company, 1990: Chapter 8.

65. Witte RS, ed. Statistics. 2d ed. Winston: Holt Rinehart, 1985: Chapter 9.

66. Valeri CR, Valeri DA, Gray A, Melarangno A, Dennis RC, and Emerson CP. Viability and function of red blood cell concentrates stored at 4°C for 35 days in CPDA-1, CPDA-2, or

CPDA-3. Transfusion 1982;22:210-216.

67. Simon ER. Adenine in blood banking. Transfusion 1977;17:317-324.

68. Bishop C, and Surgenor DM, ed. The red blood cell: a comprehensive treatise. New York: Academic Press, 1964:482.

69. Henry JB, ed. Clinical diagnosis and management by laboratory methods. 3rd ed. Philadelphia: W.B. Saunders Company, 1984:1049.

70. Devlin TM, ed. Textbook of biochemistry with clinical correlations. 3rd ed. New York: Wiley-Liss, Inc., 1992:226.

71. Mechling ML. Personal communication, Mixing PRBC prior to sampling. 12 February 1996.

APPENDIX A
DATA TABLES

Appendix A contains 10 data tables, all are organized in the same manner.

Each table contains assay values taken each day of sampling for each unit. The number indicates the pool and the letter indicates the group. There were eight pools, of three prbc units, each pool was split into three units A, B, and C. Group A units were stored at 1-6°C, except at day 6 when the units were placed in a 25°C incubator for 24 hours. Group B units were stored at 1-6°C, except at day 20 when the units were placed in a 25°C incubator for 24 hours. Group C units were stored at 1-6°C for the full 35 day storage period. The mean and standard error of the mean are given for each group in the shaded area of the tables.

Table A1. pH Values								
Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	6.99	6.79	6.59	6.52	6.47	6.47	6.44	6.42
P002A	7.01	6.85	6.61	6.53	6.48	6.47	6.44	6.43
P003A	7.00	6.81	6.59	6.50	6.44	6.43	6.39	6.37
P004A	7.01	6.83	6.61	6.53	6.47	6.46	6.44	6.40
P005A	6.98	6.80	6.59	6.51	6.46	6.44	6.41	6.38
P006A	7.01	6.86	6.64	6.55	6.50	6.51	6.47	6.44
P007A	6.99	6.79	6.58	6.52	6.47	6.46	6.43	6.40
P008A	7.00	6.84	6.60	6.51	6.46	6.46	6.42	6.38
P001B	6.99	6.81	6.78	6.63	6.55	6.46	6.43	6.42
P002B	7.01	6.82	6.79	6.65	6.57	6.46	6.42	6.40
P003B	7.00	6.81	6.77	6.62	6.52	6.42	6.38	6.37
P004B	7.01	6.84	6.81	6.66	6.56	6.45	6.42	6.40
P005B	6.98	6.80	6.77	6.61	6.54	6.44	6.40	6.38
P006B	7.01	6.86	6.83	6.68	6.58	6.48	6.44	6.42
P007B	6.99	6.79	6.76	6.62	6.53	6.45	6.41	6.39
P008B	7.00	6.84	6.80	6.64	6.54	6.45	6.40	6.37
P001C	6.99	6.81	6.78	6.63	6.56	6.54	6.49	6.44
P002C	7.01	6.83	6.80	6.64	6.56	6.55	6.49	6.44
P003C	7.00	6.81	6.77	6.62	6.52	6.50	6.45	6.39
P004C	7.01	6.83	6.80	6.68	6.56	6.55	6.48	6.44
P005C	6.98	6.81	6.77	6.62	6.54	6.53	6.47	6.42
P006C	7.01	6.86	6.83	6.67	6.57	6.56	6.49	6.45
P007C	6.99	6.79	6.76	6.63	6.53	6.53	6.48	6.42
P008C	7.00	6.84	6.81	6.66	6.56	6.55	6.47	6.42
Mean of Group A	7.00	6.82	6.60	6.52	6.47	6.46	6.43	6.40
Mean of Group B	7.00	6.82	6.79	6.64	6.55	6.45	6.41	6.39
Mean of Group C	7.00	6.82	6.79	6.64	6.55	6.54	6.48	6.43
Standard Error A	0.0043	0.0096	0.0065	0.0055	0.0060	0.0077	0.0080	0.0090
Standard Error B	0.0043	0.0081	0.0082	0.0082	0.0068	0.0056	0.0067	0.0076
Standard Error C	0.0043	0.0080	0.0082	0.0079	0.0062	0.0058	0.0046	0.0064

Table A2. Total Hemoglobin Values in g/dL								
Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	21.1	20.8	20.6	20.7	21.4	20.7	21.6	21.1
P002A	20.5	19.6	20.6	20.4	20.1	20.6	20.4	20.3
P003A	20.0	20.7	19.7	20.3	21.1	20.9	20.8	20.8
P004A	19.7	21.2	20.0	18.0	20.2	20.3	20.1	20.1
P006A	20.8	20.5	20.7	21.5	21.1	20.9	20.7	20.8
P007A	21.1	21.2	21.1	20.6	20.9	20.9	21.1	20.8
P008A	20.7	19.9	20.7	21.6	20.9	20.8	20.7	20.1
P001B	21.1	21.1	20.6	20.9	20.7	21.6	21.1	21.2
P002B	20.5	20.6	20.7	20.3	20.6	20.6	20.5	20.3
P003B	20.0	20.5	20.6	20.3	20.8	20.4	20.8	20.5
P004B	19.7	19.5	20.1	19.6	20.2	20.2	20.4	20.3
P006B	20.8	20.5	20.6	20.6	20.7	20.4	20.1	20.8
P007B	21.1	20.9	21.6	21.3	20.9	21.1	21.1	21.5
P008B	20.7	20.4	21.2	21.5	20.9	20.5	20.8	21.1
P001C	21.1	21.1	20.8	21.5	21.1	21.3	20.3	21.1
P002C	20.5	20.0	20.6	20.0	20.4	20.0	20.6	20.6
P003C	20.0	20.7	20.3	20.4	20.6	20.9	20.5	20.8
P004C	19.7	20.2	20.1	19.3	20.3	20.1	20.3	20.4
P006C	20.8	20.4	20.4	20.0	21.1	20.8	20.6	20.5
P007C	21.1	20.5	20.6	21.1	21.9	21.2	21.3	21.1
P008C	20.7	21.3	20.9	21.3	20.7	20.6	20.6	20.6
Mean of Group A	20.6	20.6	20.5	20.4	20.8	20.7	20.8	20.6
Mean of Group B	20.6	20.5	20.8	20.6	20.7	20.7	20.7	20.8
Mean of Group C	20.6	20.6	20.5	20.5	20.9	20.7	20.6	20.7
Standard Error A	0.2022	0.2318	0.1792	0.4509	0.1831	0.0837	0.1822	0.1507
Standard Error B	0.2022	0.1915	0.1835	0.2467	0.0911	0.1857	0.1405	0.1779
Standard Error C	0.2022	0.1773	0.1063	0.3066	0.2078	0.1902	0.1272	0.1063

Table A3. Plasma Hemoglobin Values in mg/dL								
Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	13.0	48.0	64.0	77.0	94	115	158	235
P002A	15.0	84.0	91.0	78.0	83	98	127	165
P003A	10.0	63.0	83.0	69.0	75	91	120	156
P004A	0.0	52.0	50.0	36.0	66	64	115	136
P006A	9.0	57.0	53.0	57.0	59	59	81	113
P007A	8.0	62.0	51.0	70.0	69	65	84	117
P008A	21.0	78.0	87.0	94.0	100	99	114	137
P001B	13.0	47.0	44.0	53.0	59	90	159	266
P002B	15.0	84.0	73.0	67.0	65	96	144	201
P003B	10.0	81.0	79.0	58.0	55	82	129	201
P004B	0.0	45.0	35.0	29.0	27	46	122	150
P006B	9.0	58.0	45.0	49.0	44	59	100	144
P007B	8.0	59.0	52.0	50.0	55	69	118	197
P008B	21.0	61.0	80.0	77.0	94	104	153	193
P001C	13.0	56.0	39.0	52.0	49	56	91	130
P002C	15.0	81.0	71.0	64.0	63	72	98	128
P003C	10.0	70.0	78.0	61.0	63	101	116	142
P004C	0.0	28.0	55.0	18.0	32	30	55	91
P006C	9.0	48.0	42.0	44.0	54	53	60	74
P007C	8.0	33.0	43.0	37.0	38	38	53	99
P008C	21.0	77.0	92.0	92.0	115	136	176	240
Mean of Group A	10.9	63.4	68.4	68.7	78.0	84.4	114.1	151.3
Mean of Group B	10.9	62.1	58.3	54.7	57.0	78.0	132.1	193.1
Mean of Group C	10.9	56.1	60.0	52.6	59.1	69.4	92.7	129.1
Standard Error A	2.4633	4.9946	6.8447	6.9066	5.6904	8.1878	9.9173	15.6581
Standard Error B	2.4633	5.7422	7.0363	5.7268	7.7367	7.9072	7.9325	15.2150
Standard Error C	2.4633	7.9265	7.7889	8.8206	10.3082	14.1655	16.6042	20.6218

Table A4. Percent Hemolysis Levels

Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	0.062	0.231	0.311	0.372	0.439	0.556	0.731	1.114
P002A	0.073	0.429	0.442	0.382	0.413	0.476	0.623	0.813
P003A	0.050	0.304	0.421	0.340	0.355	0.435	0.577	0.750
P004A	0.000	0.245	0.250	0.200	0.327	0.315	0.572	0.677
P006A	0.043	0.278	0.256	0.265	0.280	0.282	0.391	0.543
P007A	0.038	0.292	0.242	0.340	0.330	0.311	0.398	0.563
P008A	0.101	0.392	0.420	0.435	0.478	0.476	0.551	0.682
P001B	0.062	0.223	0.214	0.254	0.285	0.417	0.754	1.255
P002B	0.073	0.408	0.353	0.330	0.316	0.466	0.702	0.990
P003B	0.050	0.395	0.383	0.286	0.264	0.402	0.620	0.980
P004B	0.000	0.231	0.174	0.148	0.134	0.228	0.598	0.739
P006B	0.043	0.283	0.218	0.238	0.213	0.289	0.498	0.692
P007B	0.038	0.282	0.241	0.235	0.263	0.327	0.559	0.916
P008B	0.101	0.299	0.377	0.358	0.450	0.507	0.736	0.915
P001C	0.062	0.265	0.188	0.242	0.232	0.263	0.448	0.616
P002C	0.073	0.405	0.345	0.320	0.309	0.360	0.476	0.621
P003C	0.050	0.338	0.384	0.299	0.306	0.483	0.566	0.683
P004C	0.000	0.139	0.274	0.093	0.158	0.149	0.271	0.446
P006C	0.043	0.235	0.206	0.220	0.256	0.255	0.291	0.361
P007C	0.038	0.161	0.209	0.175	0.174	0.179	0.249	0.469
P008C	0.101	0.362	0.440	0.432	0.556	0.660	0.854	1.165
Mean of Group A	0.052	0.310	0.335	0.333	0.375	0.407	0.549	0.734
Mean of Group B	0.052	0.303	0.280	0.264	0.275	0.377	0.638	0.927
Mean of Group C	0.052	0.272	0.292	0.254	0.284	0.336	0.451	0.623
Standard Error A	0.0119	0.0279	0.0341	0.0296	0.0268	0.0395	0.0457	0.0728
Standard Error B	0.0119	0.0276	0.0332	0.0262	0.0366	0.0377	0.0361	0.0699
Standard Error C	0.0119	0.0383	0.0374	0.0412	0.0504	0.0688	0.0810	0.1001

Table A5. Plasma Sodium Levels in mmol/L								
Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	161	146	150	140	131	129	124	116
P002A	159	141	148	138	129	128	124	116
P003A	160	140	148	137	129	127	123	114
P004A	162	140	154	141	129	129	125	116
P005A	159	140	148	133	127	126	121	112
P006A	160	144	151	139	131	131	129	118
P007A	161	143	150	138	127	124	125	118
P008A	158	140	148	136	128	124	121	114
P001B	161	145	144	137	127	128	123	115
P002B	159	140	138	131	125	127	122	114
P003B	160	141	139	131	124	125	122	113
P004B	162	143	141	133	125	132	123	114
P005B	159	141	138	128	124	126	120	111
P006B	160	145	141	132	126	130	125	117
P007B	161	143	142	132	126	129	123	116
P008B	158	141	138	129	122	123	119	112
P001C	161	145	143	134	127	126	121	115
P002C	159	139	138	130	124	123	119	113
P003C	160	140	138	131	127	123	121	114
P004C	162	142	140	133	128	126	122	113
P005C	159	141	136	128	123	122	119	110
P006C	160	143	141	134	127	126	125	116
P007C	161	144	141	132	127	126	120	114
P008C	158	138	136	127	120	122	116	110
Mean of Group A	160.0	141.8	149.6	137.8	128.9	127.3	124.0	115.5
Mean of Group B	160.0	142.4	140.1	131.6	124.9	127.5	122.1	114.0
Mean of Group C	160.0	141.5	139.1	131.1	125.4	124.3	120.4	113.1
Standard Error A	0.4629	0.8183	0.7545	0.8814	0.5489	0.8814	0.9063	0.7319
Standard Error B	0.4629	0.6797	0.7892	0.9625	0.5489	1.0177	0.6665	0.7071

Standard Error C	0.4629	0.8660	0.8952	0.9342	0.9808	0.6748	0.9246	0.7662

Table A6. Plasma Potassium Levels in mmol/L

Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	3.3	22.6	22.0	34.1	42.9	44.0	52.5	61.8
P002A	3.2	25.1	21.6	33.2	41.8	43.4	51.0	59.9
P003A	3.4	26.1	23.3	33.9	44.2	45.2	53.2	62.5
P004A	3.4	27.5	23.4	33.0	44.0	42.9	54.3	61.8
P005A	3.4	26.5	24.8	35.6	42.8	44.4	54.8	62.9
P006A	3.2	22.7	21.4	31.2	38.2	40.0	49.9	56.9
P007A	3.4	25.2	22.7	33.4	38.9	38.7	50.8	56.9
P008A	3.2	27.1	24.5	35.9	42.0	42.3	51.1	59.7
P001B	3.3	22.8	25.0	37.0	45.1	45.1	53.3	63.8
P002B	3.2	25.6	27.8	38.3	46.6	45.7	52.9	64.0
P003B	3.4	25.8	28.6	38.5	47.5	46.6	54.2	65.4
P004B	3.4	25.5	28.6	39.0	48.2	50.3	56.2	68.4
P005B	3.4	25.3	29.2	39.7	46.3	45.8	56.0	65.9
P006B	3.2	22.8	26.4	36.5	42.4	42.5	51.6	60.9
P007B	3.4	25.4	27.5	37.3	43.1	42.6	49.0	59.3
P008B	3.2	26.0	29.9	40.5	44.9	45.2	53.1	63.7
P001C	3.3	23.7	25.7	37.0	46.0	47.3	54.2	65.3
P002C	3.2	25.8	28.5	38.9	47.2	48.1	54.8	66.4
P003C	3.4	26.7	29.2	38.6	43.7	47.8	54.4	64.8
P004C	3.4	26.4	28.9	38.3	46.1	47.4	57.6	67.1
P005C	3.4	26.0	29.5	39.8	46.4	48.1	57.5	67.7
P006C	3.2	24.2	26.8	35.5	42.1	43.2	53.8	62.0
P007C	3.4	24.0	27.6	37.4	43.5	45.2	51.2	62.0
P008C	3.2	28.9	31.0	41.7	46.8	48.3	54.6	66.2
Mean of Group A	3.3	25.4	23.0	33.8	41.9	42.6	52.2	60.3
Mean of Group B	3.3	24.9	27.9	38.4	45.5	45.5	53.3	63.9
Mean of Group C	3.3	25.7	28.4	38.4	45.2	46.9	54.8	65.2
Standard Error A	0.0350	0.6579	0.4500	0.5290	0.7815	0.7877	0.6291	0.8450
Standard Error B	0.0350	0.4648	0.5589	0.4862	0.7200	0.8664	0.8247	1.0092
Standard Error C	0.0350	0.6119	0.5880	0.6617	0.6573	0.6358	0.7275	0.7702

Table A7. Whole blood Glucose Levels in mg/dL								
Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	372.8	292.5	177.5	126.0	95.7	90.3	63.0	40.2
P002A	410.3	325.4	212.4	155.4	126.9	113.1	88.5	60.3
P003A	384.2	290.6	167.0	122.1	87.0	84.9	48.6	23.1
P004A	403.2	301.7	197.7	151.8	120.6	112.8	82.2	58.5
P005A	385.8	281.6	179.6	140.7	110.4	106.5	69.9	43.2
P006A	397.5	286.1	188.4	139.8	113.7	109.2	79.5	58.5
P007A	377.0	280.2	185.9	146.1	111.3	105.3	78.0	49.8
P008A	395.7	309.5	173.3	135.3	101.4	93.6	62.4	37.2
P001B	372.8	289.1	280.2	191.7	148.8	80.1	51.0	34.2
P002B	410.3	322.1	306.2	233.7	184.2	111.6	81.6	59.4
P003B	384.2	291.2	265.2	188.7	147.6	72.6	39.9	18.6
P004B	403.2	315.3	286.8	221.7	180.0	105.3	69.3	49.8
P005B	385.8	286.8	265.2	200.1	165.6	99.0	67.5	41.1
P006B	397.5	292.5	272.7	175.5	160.8	84.3	67.8	42.0
P007B	377.0	299.4	279.8	210.6	157.2	91.5	60.0	40.5
P008B	395.7	293.9	270.2	202.2	142.5	83.7	50.7	26.7
P001C	372.8	286.5	284.0	196.5	156.3	142.8	110.1	73.8
P002C	410.3	329.9	306.6	236.1	180.9	173.1	136.5	100.8
P003C	384.2	287.0	264.5	189.3	120.0	130.2	80.7	45.3
P004C	403.2	315.3	288.2	228.0	182.1	167.1	126.6	91.5
P005C	385.8	285.6	264.8	205.5	165.9	157.5	123.0	85.5
P006C	397.5	296.0	281.0	205.5	151.5	142.5	109.5	58.5
P007C	377.0	316.8	277.8	207.0	161.1	159.3	105.3	60.6
P008C	395.7	299.7	282.2	203.7	151.5	146.4	100.8	61.8
Mean of Group A	390.8	295.9	185.2	139.7	108.4	102.0	71.5	46.4
Mean of Group B	390.8	298.8	278.3	203.0	160.8	91.0	61.0	39.0
Mean of Group C	390.8	302.1	281.1	209.0	158.7	152.4	111.6	72.2
Standard Error A	4.6055	5.4668	5.1315	4.1224	4.6288	3.8328	4.6132	4.5782
Standard Error B	4.6055	4.5805	4.8081	6.6109	5.3541	4.7220	4.6943	4.5098
Standard Error C	4.6055	5.8996	4.7521	5.5038	6.9502	5.0660	6.1132	6.7134

Table A8. Red Blood Cell Morphology Scores in Percent								
Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	98.04	92.04	96.12	85.32	68.96	69.48	61.58	57.77
P002A	98.18	87.07	94.94	84.63	74.23	74.74	67.10	62.97
P003A	97.16	90.68	91.96	79.86	69.26	74.58	63.07	55.84
P004A	97.89	92.05	91.72	79.36	73.94	73.41	61.07	54.00
P005A	98.25	88.63	92.32	77.09	72.30	68.17	57.80	53.44
P006A	97.04	91.13	93.66	84.45	72.70	75.61	64.68	64.26
P007A	98.20	91.34	93.17	83.27	73.58	73.51	72.02	69.62
P008A	98.57	90.24	96.45	85.18	75.27	74.15	70.80	68.12
P001B	98.04	90.32	88.96	82.79	71.94	93.48	68.60	59.87
P002B	98.18	87.59	89.67	84.13	72.40	91.90	71.56	65.58
P003B	97.16	87.39	89.78	82.04	73.80	88.83	67.19	62.88
P004B	97.89	92.43	91.31	87.74	79.56	89.61	69.50	59.76
P005B	98.25	86.89	87.84	76.76	67.89	89.39	65.58	55.01
P006B	97.04	92.11	91.27	88.16	77.69	86.79	74.53	67.32
P007B	98.20	90.51	93.55	85.52	75.60	84.19	75.53	71.37
P008B	98.57	89.87	93.31	84.24	72.83	87.50	77.41	70.92
P001C	98.04	88.61	90.81	82.27	71.27	72.14	63.54	63.82
P002C	98.18	84.68	89.46	80.87	69.13	74.83	64.22	65.86
P003C	97.16	89.49	89.50	82.34	77.19	79.36	66.42	65.73
P004C	97.89	92.29	89.13	84.25	80.76	79.46	67.52	60.26
P005C	98.25	88.30	88.78	76.74	65.98	71.69	62.73	56.60
P006C	97.04	89.95	90.44	83.72	76.76	82.24	70.16	69.24
P007C	98.20	86.56	91.46	82.55	72.99	75.75	74.90	71.87
P008C	98.57	91.48	95.20	87.47	72.33	78.06	74.06	72.48
Mean of Group A	97.92	90.40	93.79	82.40	72.53	72.96	64.77	60.75
Mean of Group B	97.92	89.64	90.71	83.92	73.96	88.96	71.24	64.09
Mean of Group C	97.92	88.92	90.60	82.53	73.30	76.69	67.94	65.73
Standard Error A	0.1911	0.6146	0.6550	1.1186	0.8129	0.9429	1.7408	2.2450
Standard Error B	0.1911	0.7557	0.7165	1.2772	1.2861	1.0290	1.4990	2.0388
Standard Error C	0.1911	0.8796	0.7313	1.0787	1.6844	1.3197	1.6564	1.9481

Table A9. Whole Blood Lactate Levels in mg/dL								
Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	22.1	122.0	237.0	274.0	293.0	286.0	334.0	330.0
P002A	16.8	102.5	217.0	247.0	280.0	257.0	326.0	320.0
P003A	19.0	109.5	204.0	262.0	283.0	296.0	340.0	366.0
P004A	16.5	111.5	198.0	253.0	283.0	289.0	318.0	354.0
P005A	17.2	106.0	202.0	238.0	272.0	283.0	320.0	348.0
P006A	16.1	90.5	393.0	233.0	258.0	256.0	290.0	320.0
P007A	16.5	109.0	201.0	262.0	273.0	274.0	302.0	338.0
P008A	13.3	95.0	201.0	262.0	268.0	280.0	318.0	336.0
P001B	22.1	120.5	134.5	193.0	235.0	272.0	336.0	286.0
P002B	16.8	101.0	124.5	179.0	208.0	272.0	326.0	332.0
P003B	19.0	110.5	120.0	195.0	235.0	288.0	354.0	362.0
P004B	16.5	100.5	107.5	173.0	228.0	290.0	316.0	358.0
P005B	17.2	126.5	113.0	166.0	215.0	280.0	308.0	348.0
P006B	16.1	90.0	111.5	161.0	211.0	276.0	298.0	337.0
P007B	16.5	109.5	119.5	188.0	223.0	290.0	330.0	337.0
P008B	13.3	102.5	111.5	185.0	221.0	292.0	336.0	350.0
P001C	22.1	114.5	136.5	186.0	238.0	232.0	292.0	352.0
P002C	16.8	127.0	119.0	175.0	216.0	219.0	266.0	284.0
P003C	19.0	107.0	124.5	192.0	239.0	249.0	292.0	334.0
P004C	16.5	103.0	107.0	170.0	222.0	229.0	282.0	320.0
P005C	17.2	99.5	116.0	156.0	216.0	214.0	266.0	296.0
P006C	16.1	103.0	100.0	163.0	213.0	223.0	266.0	295.0
P007C	16.5	106.0	116.5	184.0	219.0	244.0	264.0	298.0
P008C	13.3	94.5	106.0	173.0	207.0	229.0	292.0	297.0
Mean of Group A	17.2	105.8	231.6	253.9	276.3	277.6	318.5	339.0
Mean of Group B	17.2	107.6	117.8	180.0	222.0	282.5	325.5	338.8
Mean of Group C	17.2	106.8	115.7	174.9	221.3	229.9	277.5	309.5
Standard Error A	0.8945	3.4859	23.5022	4.8896	3.8161	5.1372	5.7415	5.7446
Standard Error B	0.8945	4.1520	3.1053	4.4118	3.6401	2.9940	6.2764	8.4065
Standard Error C	0.8945	3.5392	4.0926	4.2780	4.0697	4.2022	4.6866	8.2894

Table A10. Whole Blood ATP Levels in µmole/g Hgb								
Unit Number	Day 0	Day 6	Day 7	Day 14	Day 20	Day 21	Day 28	Day 35
P001A	4.4	5.2	4.6	3.3	2.3	2.6	1.4	1.0
P002A	4.7	5.3	5.2	3.6	2.7	2.8	2.1	1.5
P003A	4.8	4.7	5.1	3.0	2.5	2.5	1.9	1.4
P004A	4.3	4.8	4.2	2.7	2.3	2.3	1.7	1.2
P005A	3.7	4.6	4.5	3.0	2.7	2.5	1.9	1.4
P006A	3.8	3.3	4.5	2.8	2.7	2.5	1.8	1.4
P007A	4.5	5.7	5.1	3.7	3.2	3.2	2.2	1.7
P008A	4.9	5.1	5.1	3.5	3.1	2.9	2.1	1.6
P001B	4.4	5.2	5.2	4.5	4.0	2.9	1.7	1.0
P002B	4.7	5.0	5.0	5.2	3.9	3.5	2.2	1.4
P003B	4.8	5.2	4.6	4.9	2.9	3.1	2.0	1.3
P004B	4.3	4.3	3.4	4.5	3.0	2.9	1.8	1.2
P005B	3.7	5.0	4.9	4.7	4.1	3.1	1.7	1.4
P006B	3.8	4.4	4.6	4.5	4.1	3.0	1.8	1.3
P007B	4.5	6.0	5.5	5.1	4.7	3.4	2.1	1.7
P008B	4.9	5.0	5.8	5.0	5.0	3.5	1.9	1.6
P001C	4.4	4.9	5.4	4.4	3.7	4.1	3.1	2.2
P002C	4.7	5.2	5.4	5.3	3.6	4.5	3.5	2.4
P003C	4.8	5.0	5.0	4.8	3.3	4.0	3.1	2.2
P004C	4.3	4.3	3.5	4.4	3.3	3.7	2.9	2.1
P005C	3.7	4.6	5.1	4.4	4.2	4.1	2.7	2.1
P006C	3.8	4.5	4.0	4.4	4.2	3.9	2.9	2.3
P007C	4.5	5.7	5.5	4.9	4.7	4.6	3.2	2.9
P008C	4.9	4.9	5.0	5.2	4.8	4.6	3.3	2.7
Mean of Group A	4.4	4.8	4.8	3.2	2.7	2.7	1.9	1.4
Mean of Group B	4.4	5.0	4.9	4.8	4.0	3.2	1.9	1.4
Mean of Group C	4.4	4.9	4.9	4.7	4.0	4.2	3.1	2.4
Standard Error A	0.1563	0.2535	0.1342	0.1336	0.1172	0.1017	0.0915	0.0779
Standard Error B	0.1563	0.1856	0.2569	0.1018	0.2577	0.0901	0.0655	0.0778
Standard Error C	0.1563	0.1552	0.2563	0.1346	0.2085	0.1202	0.0895	0.1034

APPENDIX B
ANOVA TABLES

Appendix B contains 9 ANOVA tables, all are organized in the same manner.

Each table contains the single factor ANOVA for the analytes tested. There were eight pools, of three prbc units, each pool was split into three units A, B, and C. Group A units were stored at 1-6°C, except at day 6 when the units were placed in a 25°C incubator for 24 hours. Group B units were stored at 1-6°C, except at day 20 when the units were placed in a 25°C incubator for 24 hours. Group C units were stored at 1-6°C for the full 35 day storage period.

The first section of each table compares the day 35 means of groups A, B, and C. The second section compares the means of A and B, at day 28 with the mean of group C at day 35.

The following abbreviations were used:

SS	Sum of the squared deviations
df	Degrees of freedom
MS	Mean square

Table B1 ANOVA for pH						
Anova: Single Factor	**pH Day 35 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 35	8	51.216	6.402	0.000642		
B Day 35	8	51.147	6.393375	0.000463		
C Day 35	8	51.438	6.42975	0.000333		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00578	2	0.00289	6.029842	0.008524	3.466795
Within Groups	0.010065	21	0.000479			
Total	0.015846	23				
Anova: Single Factor	**pH Day 28 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 28	8	51.425	6.428125	0.000515		
B Day 28	8	51.311	6.413875	0.000356		
C Day 35	8	51.438	6.42975	0.000333		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.001221	2	0.00061	1.521879	0.241423	3.466795
Within Groups	0.008421	21	0.000401			
Total	0.009642	23				

Table B2. ANOVA for Supernatant Hemoglobin						
Anova: Single Factor	**Supernatant Hemoglobin Day 35 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A day 35	7	1059	151.2857	1716.238		
B day 35	7	1352	193.1429	1620.476		
C day 35	7	904	129.1429	2976.81		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	14789.43	2	7394.714	3.51375	0.051487	3.554561
Within Groups	37881.14	18	2104.508			
Total	52670.57	20				
Anova: Single Factor	**Supernatant Hemoglobin Day 28 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A day 28	7	799	114.1429	688.4762		
B day 28	7	925	132.1429	440.4762		
C day 35	7	904	129.1429	2976.81		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1302	2	651	0.475673	0.62906	3.554561
Within Groups	24634.57	18	1368.587			
Total	25936.57	20				

Table B3. ANOVA for Percent Hemolysis						
Anova: Single Factor	**Hemolysis Day 35 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A day 35	7	5.14053	0.734361	0.037084		
B day 35	7	6.487548	0.926793	0.034162		
C day 35	7	4.361462	0.623066	0.070174		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.330554	2	0.165277	3.506115	0.05177	3.554561
Within Groups	0.848514	18	0.04714			
Total	1.179069	20				
Anova: Single Factor	**Hemolysis Day 28 Versus day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A day 28	7	4.21729	0.60247	0.014768		
B day 28	7	4.466556	0.638079	0.009147		
C day 35	7	4.361462	0.623066	0.070174		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.004474	2	0.002237	0.071334	0.931413	3.554561
Within Groups	0.56453	18	0.031363			
Total	0.569004	20				

Table B4. ANOVA for Plasma Sodium						
Anova: Single Factor	**Sodium Day 35 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 35	8	924	115.5	4.285714		
B Day 35	8	912	114	4		
C Day 35	8	905	113.125	4.696429		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	23.08333	2	11.54167	2.667125	0.092861	3.466795
Within Groups	90.875	21	4.327381			
Total	113.9583	23				
Anova: Single Factor	**Sodium Day 28 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 28	8	992	124	6.571429		
B Day 28	8	977	122.125	3.553571		
C Day 35	8	905	113.125	4.696429		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	540.75	2	270.375	54.72651	4.69E-09	3.466795
Within Groups	103.75	21	4.940476			
Total	644.5	23				

Table B5. ANOVA for Plasma Potassium						
Anova: Single Factor	**Potassium Day 35 Vs Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 35	8	482.4571	60.30714	5.712536		
B Day 35	8	511.3429	63.91786	8.148557		
C Day 35	8	521.5286	65.19107	4.745711		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	102.6962	2	51.34812	8.278927	0.002233	3.466795
Within Groups	130.2476	21	6.202268			
Total	232.9439	23				
Anova: Single Factor	**Potassium Day 28 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 28	8	417.6	52.2	3.165714		
B Day 28	8	426.3	53.2875	5.44125		
C Day 35	8	521.5286	65.19107	4.745711		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	831.0549	2	415.5275	93.35825	3.55E-11	3.466795
Within Groups	93.46872	21	4.450892			
Total	924.5237	23				

Table B6. ANOVA for Whole Blood Glucose						
Anova: Single Factor	**Glucose Day 35 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 35	8	370.8	46.35	167.6829		
B Day 35	8	312.3	39.0375	162.7055		
C Day 35	8	577.8	72.225	360.5593		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4865.063	2	2432.531	10.56172	0.00067	3.466795
Within Groups	4836.634	21	230.3159			
Total	9701.696	23				
Anova: Single Factor	**Glucose Day 28 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 28	8	572.1	71.5125	170.2527		
B Day 28	8	487.8	60.975	176.2907		
C Day 35	8	577.8	72.225	360.5593		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	634.9575	2	317.4788	1.346956	0.28159	3.466795
Within Groups	4949.719	21	235.7009			
Total	5584.676	23				

Table B7. ANOVA for Red Blood Cell Morphology						
Anova: Single Factor		**Red Cell Morphology Day 35 Versus Day 35**				
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 35	8	486.02	60.7525	40.31848		
B Day 35	8	512.71	64.08875	33.25258		
C Day 35	8	525.86	65.7325	30.36071		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	103.021	2	51.5105	1.486855	0.248933	3.466795
Within Groups	727.5224	21	34.64392			
Total	830.5434	23				
Anova: Single Factor		**Red Cell Morphology Day 28 Versus Day 35**				
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Pool A day 28	8	518.12	64.765	24.24246		
Pool B day 28	8	569.9	71.2375	17.97639		
Pool C day 35	8	525.86	65.7325	30.36071		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	195.0249	2	97.51245	4.030575	0.033	3.466795
Within Groups	508.0569	21	24.19319			
Total	703.0818	23				

Table B8. ANOVA for Whole Blood Lactate						
Anova: Single Factor	**Lactate Day 35 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 35	9	3051	339	231		
B Day 35	9	3048.75	338.75	494.6875		
C Day 35	9	2785.5	309.5	481		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5177.625	2	2588.813	6.436163	0.005783	3.402832
Within Groups	9653.5	24	402.2292			
Total	14831.13	26				
Anova: Single Factor	**Lactate Day 28 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 28	8	2548	318.5	263.7143		
B Day 28	8	2604	325.5	315.1429		
C Day 35	8	2476	309.5	549.7143		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1029.333	2	514.6667	1.368101	0.276366	3.466795
Within Groups	7900	21	376.1905			
Total	8929.333	23				

Table B9. ANOVA for ATP						
Anova: Single Factor	**ATP Day 35 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 35	8	11.2	1.4	0.048571		
B Day 35	8	10.9	1.3625	0.048393		
C Day 35	8	18.9	2.3625	0.085536		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	5.140833	2	2.570417	42.25342	4.35E-08	3.466795
Within Groups	1.2775	21	0.060833			
Total	6.418333	23				
Anova: Single Factor	**ATP Day 28 Versus Day 35**					
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
A Day 28	8	15.1	1.8875	0.066964		
B Day 28	8	15.2	1.9	0.034286		
C Day 35	8	18.9	2.3625	0.085536		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.1725	2	0.58625	9.41587	0.001205	3.466795
Within Groups	1.3075	21	0.062262			
Total	2.48	23				